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## Letter from the Guest Editors

### **NANOTECHNOLOGY AND BIOSENSORS: IMPACT IN HEALTH, ENVIRONMENT, SAFETY AND SECURITY OF ALBANIA**

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Guest Editors of the Special issue of JNTS Editorial Board

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Nanotechnology in general and particularly its building block nanomaterials are showing to be of great interest in developing a new generation of sensors and biosensors. Such emerging technology and materials have attracted scientists all over the world and have shown to be very interesting for the research community in Albania working in health, environment, safety and security beside other industries. The objective of this JNTS special issue is to show the current status of this emerging field of research in Albania following also a three days' Workshop- ALBNANO 2016 - "Nanotechnology and biosensors, impact in health, environment, safety and security of Albania" that was held in Tirana from 29 to 31 of May 2016 as a close cooperation with the Catalan Institute of Nanoscience and Nanotechnology, Spain (ICN2) and financially supported from the Faculty of Natural Sciences at University of Tirana, Academy of Sciences of Albania and the Spanish Embassy in Tirana.

ALBNANO workshop aimed to show advances of the scientific research in general in the field of nanotechnology and biosensors in Albania and particularly of their applications in environment protection, health, food safety and control. Speakers from various universities in Albania such as from University of Tirana, Agricultural University of Tirana, Polytechnic University of Tirana and the Medical University of Tirana showed their experience in this field over the years. The supporting policies/ strategies to

the science and technology in the country were presented from representatives of the Ministry of Education and Sports and the Ministry of Innovation in addition from the Academy of Science of Albania. Guest speakers from Spain, the Director of CERCA (<http://cerca.cat/>), Dr. Lluís Rovira, and Director of ICN2 (<http://icn2.cat/>), Prof. Pablo Ordejon from Barcelona, Spain made overviews of the research and development strategies in their country and respective institutions. Prof. Giuseppe Palleschi, from Tor Vergata University in Rome (Italy) in addition to over 70 participants with oral /poster presentations from more than 10 institutions such as the University of Tirana, Polytechnic University of Tirana, Epoka University, University of Pristina, Tetovo University, Academy of Sciences Tirana, University Tor Vergata (Rome, Italy), ICN2-Spain, CERCA (Barcelona, Spain), ICREA (Barcelona, Spain), Ministry of Innovation, and co-workers from other universities in Germany, Spain, Italy, the Netherlands, Turkey etc. also exchanged their expertise in nanotechnology, nanomaterials and nanobiosensors development and applications. Most of the presented works were carried out by Albanian scientists in collaboration with research groups and laboratories around Europe and other countries.

In addition to works presented at ALBNANO workshop, this JNTS special issue also includes other contributions from other Albanian authors and their collaborators from other countries related to recent developments in nanotechnology and biosensors. The issue covers from research and applications in cutting edge nanomaterials (ex, nanoparticles, carbon nanotubes, graphene etc.) to natural occurring nanomaterials in Albanian territories, the various nano- characterisation tools, smart nanomaterials, nanodevices (nanosensors and nanobiosensors) building and applications in various areas such as health, environment and other industries. As guest editors we are convinced that this modest issue dedicated to nanotechnology and nanobiosensors will be helpful for JNTS readers interested in the area. It will be helpful to enhance the collaboration between Albanian and international researchers working in complementary and synergetic fields and willing to use nanotechnology and overall build and apply sensors and biosensors for various applications.

The guest editors would like to thank all the contributors for their efforts to show what they are doing in the field of nanotechnology and biosensors, all the Albanian institutions and the Embassy of Spain in Tirana, and particularly the Ambassador Ms. Silvia Cortes Martin, for the given support to ALBNANO workshop organized in Tirana.

## **NANOTECHNOLOGY AND BIOSENSORS – A KEY FEATURE OF SCIENTIFIC DEVELOPMENT AND THEIR APPLICATIONS AREAS IN ALBANIA**

**Salvatore BUSHATI**

Head of the Section of Natural and Technical Sciences  
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The Albanian Academy of Sciences is one of the most important scientific institutions in the country. Its priority functions are:

- collaborating with research and education institutes of scientific excellence,
- introducing new initiatives in the area of research and education to support current needs of the country,
- helping find solution to many crucial issues concerning the development of the country by providing high state institutions with appropriate advice and expertise,
- running of scientific activities on a wide range of topics related to science, both at a national and international level,
- creating *ad-hoc* commissions of highly regarded science priorities on research and development,
- running of competitions and awards in the form of *medals* and *prizes* for outstanding achievement in the area of research and science etc.,
- establish strategies to direct the future of related science areas,
- run of and coordinate science activities and oversee their developing process,
- investigate on science needs and establish the skeleton of the future goals of science,
- advisory work relates to the recommendations made on new scientific structures; establishing specialized interdisciplinary research groups and commissions about issues of a particular interest involving at the same time even outside specialists,
- propose and organize congresses, conferences, symposiums and science seminars at national and international level and,
- publish related scientific publications: books, monographs & reports periodicals journals on RTD & I, related to the priorities areas, etc.

*The priorities of the Section of Natural and Technical Sciences have been* in the last five years in full accordance with the National Strategy for Research, Technology and Innovations and with the Government needs in the following fields as:

- information and communication technology and its application;
- health; management system, primary services, etc.;
- water and evaluation and rearrangement of the aquatic regimes in lake, rivers and lagoons;
- energy with the priority renovation ones: hydro, soil, geothermal and biomass;
- agriculture, food, security;
- environment, evaluation, conservation of natural ecosystems;
- forest and their problematic of management;
- biodiversity and quality of the food;
- nanotechnology related to the environment, health etc.;
- application of new technologies in the area of biotechnology biosafety and food;
- civil emergencies, preparedness and security, etc.

**As research and technologic development and innovation are considered to be** a key feature of the development of the country, continuous research and involvement of innovative technologies would be of crucial need. Consequently, investments and financial support from the government / governmental bodies are needed.

On the institutional context, despite a number of important initiatives undertaken at national level during the last two decades, the research and innovation sector is still characterized by a legacy of either unfinished or everlasting reforms.

National innovation system is characterized by generally low scientific performance and weak 'linkage' with the economy (in both technology transfer and collaboration). In a nutshell, their contribution to economic growth and job creation is too low.

On the other hand, data report that the scientific performance remains low.

The limited supply of 'inputs' to scientific research is one widely recognized cause for such performance for many reasons.

Available public funding for research declined and became more uncertain in recent years and reduced funding is in part an outcome of more stringent fiscal policies.

A large number of highly qualified researchers emigrated in the mid-1990s and research infrastructure has significantly deteriorated. These trends, in turn, result from different processes. The lack of appropriate maintenance and upgrade of the research infrastructure stems in part from less (and more

uncertain) public funding. Also a large diversification of the ‘science-base’ further deepens the fragmentation of already scarce resources. The past political instability, poor funding and inadequate research infrastructure encouraged a highly mobile labour force to emigrate and young scientists will likely continue to leave (and expats are unlikely to come back) without better research conditions and more transparent, merit-based career opportunities.

The limited demand for knowledge from the enterprise sector is often cited as the main cause for lack of research commercialization and collaboration. Economic reforms had extinguished (or significantly reduced).

Technology transfer is practically non-existent and collaboration between public research organizations and industry is ‘fragile’ at best. The technology transfer and collaboration are critical for achieving economic impact of public R&D; collaboration happens mainly at the individual level, driven by occasional opportunities and short-term objectives. Patent applications, a prerequisite for licensing, have been stagnating or decreasing and are notably behind other comparable economies. Spinoff companies are at the low level. In this condition robust industry-science interactions are essentially missing; even more so given that the vast majority of R&D spending in the country is performed by the public sector.

This structural factor would explain the level of business R&D expenditures and innovation performance that are lower than and in the countries of the region.

Policy discussions have paid only limited attention to the impact of weak institutional framework on innovations. Policy disincentives (both economic and non-economic) divert the behaviour of individuals and organizations away from desired directions as:

- rigid salary structures;
- job classification, and promotion rules severely;
- constrain incentives for good researcher performance;
- regulatory frameworks and funding practices discourage;
- research excellence and,
- commercialization and collaboration.

Meritocracy is weak as illustrated by the limited use of performance evaluation. Research funding is often made on a head-count basis (institutional block grants) rather than on a project performance basis (competitive grants or scientific excellence).

Career promotion is often based solely on the number of publications rather than their impact factors, and there is no recognition of technology transfer activities.

Policy frameworks regarding the ownership and commercialization of results from public-funded research (e.g. Intellectual Property Rights) are

weak or unclear, with the resulting uncertainty about the expected benefits to institutions and researchers (e.g. revenue and royalty sharing) from engaging in technology transfer activities.

Support for the development of technology transfer offices is quite scarce and government support tends to emphasize investments in physical infrastructure (such as science parks) for which the existing demand is often unclear).

*A Reform on National Research, Technology and Innovation System* is of an immediate importance.

During to the last two decades, a number of reforms and programs have run to improve the performance of the research and innovation sector. From the Government, Academy of Sciences and Ministry of Education and Sciences have provided incentives to encourage the return of national researchers, including visiting and post-doc fellowships, and installation grants removing barriers to mobility. Those program stent to show, however, a less clear track record. Albania's 2010-2015 Strategy proposes the creation of 4-5 Centres of Excellence in Science (ACES). As part of the R&D Infrastructure Investment Initiative, the country have been planned to invest in a number of research fields, including nano-science and new materials – an area of formal interest of all other countries in the region.

At the level of institutional development, important steps have been taken recently.

In Albania, the reforms have been undertaken to improve the strategic governance of research and innovation policies: has been created ARTI to support, monitor and assess science, technology and innovation programs and projects, using funds from the state budget, international programs and the private sector intended for R&D and innovation.

***For the Reform Agenda – Snapshots a new National Strategy on Research, Technology Development and Innovations (NSRTD&I) and a new structure of the Research, Technology Development and Innovation System (RTD&IS) would be needed in addition to the legislation for the RTD&I and for the institutions — a fundamental part of the system.***

In this context within a systemic framework (“innovation ecosystem”), different actors and resource capabilities as well as appropriate policy frameworks are required to allow new ideas to flourish and be effectively transformed into new economic competences. For that *Snapshots* will be effective.

An **enabling environment for firm innovation** requires a strong and clear regulatory framework (e.g. intellectual property and foreign direct investment laws) and an institutional set-up that ensures contract enforcement, encourages risk-taking investment, as well as a tax code promoting business R&D and venture creation, among other things. To **make research and**



**education systems more competitive**, with increased autonomy and transparency in budget allocation, and with an incentive framework for researchers that encourages both research excellence and academic entrepreneurship, including a clearer definition of intellectual property rights resulting from public funded research.

An *Action Plan* would involve:

- **Research Excellence Fund.** Its main objective will be to strengthen the level of research and its quality through competitive grant funding for research, in key scientific domains and connecting local scientists with the large Diaspora of emigrated scientists and entrepreneurs.
- **Centres of Excellence Program.** These research centres will focus on research areas with comparative regional advantage to enable a regional “smart specialization”. It will also be an instrument to foster research training and promotion of joint research. The centres will host and manage research networks in their corresponding fields. They will provide research training and promote joint (intra-regional) research.
- **Technology Transfer Facility.** A regional technology transfer facility will help manage the regional research pool and outcomes and their transfer to industry. This facility will assist national technology transfer offices (TTOs) in the development of skills and facilitate access to technology transfer services.
- **Innovation Facility.** This facility would work with local and international investors in the financing of regional start-up companies; provide training and business support to particularly start-ups, and serve as a bridgehead for deal flow in the region through a variety of business development services and instruments.

It is very important to determine the priority fields on RTD&I of the country. The goals of the Priority Research Fields are to combine strong research capacity with economic potential.

*Nanotechnology & Biosensors* is one from the *Priority Research Fields*, which can be with the large contribution to the development needs of the country in Priority Research Fields.

In this point of view, related to this Conference about the *application of the nanotechnology and biosensors as a necessity of scientific development in Albania*, in:

- *Agriculture and foods and biosecurity,*
- *Environment,*
- *Health and medicine technologies,*
- *Aquaculture and marine culture fields,*
- *Fields of biological sciences and chemistry, etc.*

According to the current infrastructure, **Research networks** are very important and effective.

They are responsible, among others, for the development and management of research networks in such corresponding selected research areas. Research networks can potentially enable voluntary association of researchers facilitating knowledge and information exchange through data platforms and other cyberspace infrastructure; and promoting intra-regional research collaboration and training.

**Research infrastructure** should be managed for collective use. Overall, the centres should operate in line with international good practices for the management of joint research facilities. The impact of RTD&I will be: more international orientated, increasing the collective access to knowledge and physical infrastructure capacity. At the same time integration of education, training and research will be more effective.

Research infrastructure will stimulate the dynamic turnover of researchers with equal opportunities for participating countries.

According the National Strategy of RTD&I, Albania planned to start up 4 to 5 '**Centres of Excellence**'; this initiative is in harmony with the Regional Strategy of the Research for the Innovations in the Western Balkan Countries too (approved at 2013 in Zagreb, Croatia from the ministers of the sciences and technology of the WBC).

The 'Centres of Excellence' will be act as connectivity-hubs with a network of researchers spread across the region in the selected research fields.

It is also envisaged that kind selected centres of excellence will provide contributing to a more balanced distribution of human capital, with high records, in the country and region. Upgrading the infrastructure where needed should also be considered.

Expected impact of Centre of Excellence will be:

- training and support to the formation of young scientists from lagging organizations (short and medium-run fellowships; post-doctorates and other research opportunities);
- increased research excellence by targeting areas of high relevance in the country development and regional too;
- increased number of researchers in key strategic areas;
- increased number of national and international publications;
- expanded presence of scientists from Western Balkan countries with the currently lowest levels of human capital and infrastructure for science;
- increased participation in Horizon 2020 and integration to ERA;

At the same time we can count outcomes from research excellence base & application on the Nanotechnology and biosensors like:

- more powerful national innovation system;
- high scientific performance;

- contribution to economic growth;
- decreasing the number young scientists emigration to encourage the return of national researchers, merit-based career opportunities and increasing the Diaspora contribution;
- increasing demand for knowledge from the enterprise sector, and lack of research commercialization and collaboration;
- increasing the significance of research-intensive industries and demand for knowledge by the 'structure' of the national economy and more;
- increasing the level of business R&D expenditures and innovation performance in the countries with impact and in the region;
- increasing technology transfer and collaboration between public research organizations and industry;
- increasing the Patent applications, according the needs of economy;
- more robust industry-science interactions;
- increasing the job creation number;
- Increasing the spinoff companies, etc.

We have both the intellectual capacities and human resources capacities, as a first step, to start applying properly Nanotechnology and Biosensors in different science areas. Here, building up a Centre of Excellence with operation in an international level in the framework of Horizon 2020 Program would be important.



## **THE MAIN SCIENTIFIC HUB IN SOUTH-WESTERN EUROPE: THE CASE OF CERCA R&D INSTITUTES, BARCELONA, SPAIN**

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### **ABSTRACT**

This paper reviews the recent creation and activity of the 41 CERCA centres existing today, in the area of Barcelona. They all have their own legal status under the umbrella of the CERCA Institution. I-CERCA plays a control and coordinating role including evaluation, selection of directors, merging CERCA centres, national and international promotion, technology transfer benchmarking, etc. CERCA centres are built on a basis of institutional collaboration with Universities and promoted by the Government of Catalonia. The outputs measured by ERCs granted, coordination of H2020 projects, HRS4R awards, highly cited papers, etc. are significant and provide information for measuring the accountability of public money invested in CERCA. The comparison of their scientific production (and impact) in 2012-2014 with CNRS, Max Planck and CSIC is surprising! CERCA's contribution to the expanding Catalan Science is crystal-clear. Still important challenges for the next years, specially linked to the achievement of results linked to innovation activity.

*Keywords:* Science policy, Centres of R&D, CERCA, accountability, HRS4R, ERC grants, scientific production, technology transfer, evaluation

### **1. INTRODUCTION TO CATALONIA R&D**

Catalonia is a small country of 7.5 million inhabitants in the South-West of Europe embedded within the Spanish State. Its capital city is Barcelona. Catalonia is a country of entrepreneurs with a solid industrial tradition. The country is open to science, business and open to the world. There are several examples of Catalans that deserve certain recognition for his creativity too. For instance Antoni Gaudí, was the architect that designed, among other outstanding projects, the Sagrada Familia. And at the same time we can show a few examples of excellent management as the Football Club Barcelona.

We like to tell that we Catalans share the skills of creativity and efficient management when we refer to Science and Science Policy.

Catalonia is 6% of Spanish territory with only 16% of the Spanish Population. However the GDP is 20% of Spain. The investment in R&D is 23% and the Pharmaceutical Industry is 47% of the Spanish one. In 2016, Barcelona leads the top 10 major cities of European cities of the future. And the area of Barcelona was in 2015 the fourth European region in terms of foreign investment attraction.

Since the year 2000, Catalonia has developed its own successful research ecosystem. Some of the key factors are the sustained funding from Catalan Government, the research Centres autonomy, the implementation of independent evaluation at different levels, the high performance in attracting EU funds, the open talent recruitment and the establishment of professional Government agencies for support, management and evaluation of research grants and institutions. Up to date one of the achievements is that Catalonia holds in 2014 1,3% of the World share in scientific production (scientific papers), according to the Web of Science data. According to this, the most relevant figure is that highly cited papers grow much faster than global scientific production in the country. Catalonia as a whole has doubled in the last 15 years its scientific production share to the world. And in terms of Highly Cited papers (HCPs) the increase in the World share has been 5-fold approximately.

The Catalan scientific production is mainly produced in the disciplines of Biomedicine, followed by Sciences, and in a much lower ratio Engineering, Social Sciences and Humanities. This profile is the typical one of the western countries advanced in science, since a very high importance and priority is given to Health related research projects and publications.

In terms of European Union projects Catalonia is performing in a very competitive way. In fact, the more challenging a call is, the better Catalonia's researchers performs. In the European Research Council (ERC) grants, the most restrictive and difficult EU call, with less than 10% of success, Catalonia ranks 4<sup>th</sup> in Europe just behind Switzerland, Israel and the Netherlands, with 27 grants per million inhabitants. Again, at the initial stage of Horizon 2020 EU research Programme Catalan research institutions have attracted 73% more funds than in the first two years of the FP7 (the prior EU Programme) with a total amount of 226,8 Million Euros awarded, performing in a similar way like Austria.

Finally is worthy to remark that a wide range of cutting-edge facilities that support the R&D system are present in the country, and bring together the players of the value chain: universities, research centres and companies. Among them there are the ALBA Synchrotron, The Barcelona Super Computing Centre, and several research platforms from universities and research centres.

### **The CERCA research Centres**

In appendix 1 all CERCA research Centres are listed. In the challenging Catalan R&D context the CERCA institutes are an asset. Some of them were created 20 years ago and some others were recently created at the beginning of the new century. However all of them were grouped under the CERCA Programme to create a new structure complementing the traditional role in research of the universities.

The scientific policy regarding CERCA centres in Catalonia has been developed under a framework of political consensus and institutional collaboration especially with Universities and Hospitals, searching win-win synergies for the country. The generosity of stakeholders and the rigorous task of the managers at different levels have perfectly complemented the key role of researchers. One step forward has been done

CERCA Centres are affected by a 2011 Catalan law. The law defines the perimeter of institutions gathered under the brand CERCA, and also establishes the main features of the CERCA system. According to this, the CERCA institutes have an independent legal status. Many of them are foundations, and they must have the ambition to become scientifically excellent in a context of international orientation. These institutes choose the Director and main researchers after a rigorous process of selection with external evaluation, usually delegated to the CERCA Institution. They mostly follow private sector rules to self-manage Human resources and economic performance. All of them are located in University campuses but they are not University Centres. They are there to collaborate and to create the right atmosphere of scientific discussion and to support top lecturing in higher education. This relationship with universities and other research institutions allows the adscription of University professors and other researchers to CERCA Centres. At the governance level the CERCA Institutes have, each of them, a board of trustees with a strong authority where Catalan Government, CERCA institution and other agents are represented.

Scientifically, the existence of a Scientific Advisory Board with outstanding international members is compulsory to address properly the scientific decisions to be taken by the board of trustees, advising the task of the Director. Catalan CERCA Centres share a deep focus on both, scientific top issues and knowledge and technology transfer. They are expected to boost national economy, the part based in technologies, creating spin-off companies and licencing new technologies.

CERCA initially was a programme of the Catalan Government mainly to fund R&D centres. However when the CERCA Institution (I-CERCA) started its endeavour in 2010, as the Agency devoted to support CERCA centres, some other strategic issues arose. I-CERCA monitored and externally evaluated the activity of the CERCA centres from a holistic point of view,

analysing the scientific performance, the knowledge and technology transfer, the recruitment of researchers, the administrative management and the scientific dissemination and impact. The first international evaluation exercise was held in 2012-2013. Now, again in 2016-2018 CERCA centres are being re-evaluated. Now the assessment is expected to classify CERCA centres in four categories of performance. Low performance will be associated to executive consequences like thinking over the continuity of the centre.

In addition I-CERCA has worked to achieve a certain level of recognition for CERCA at national and international level, and has promoted technology transfer activities through the CERCA KTT Commission. This Agency has also managed the SUMA programme, supported by the Catalan Government, oriented to reduce the number of centres through merging them in a bottom-up exercise complemented by economic incentives. CERCA started in 2010 with 47 centres and now only 41 are still active. It's important to note that almost no scientific capacity has been lost. Merges include all research groups in the resulting centre. Like this, some emblematic institutes have disappeared (IG, CREAL, CRESA, IMPPC, CRESIB ...) being merged with other ones, and creating ambitious scientific platforms to fight for relevant EU projects. Finally, I-CERCA has been responsible, in connection with the Scientific Advisory Board of each CERCA centre, for the selection of Directors at the CERCA centres. Fresh air, avoiding in-breeding in open, merit-based international calls has been the result.

But may be the main question is how do CERCA institutes perform in the European Union?

Such a question can be answered through the comparison of the scientific production of CERCA with the French system of research Centres "CNRS", the German "Max Planck" institutes, and the Spanish centres of "CSIC".

The scientific performance is heterogeneous but outstanding. In the period 2012-2014 all CERCA centres have published over 20.000 papers in WoS journals, and 1.116 papers have evolved as highly cited. The ratio of foreign authors in this scientific production is 53%. And only 9.2% of the articles have not been cited yet. The Relative Citation Impact (RCI) related to citation obtained comparing to other papers at the same journal and year is 2.5, therefore two and half times over the average citation. Comparing CERCA to CNRS, Max Planck and CSIC for the same time-window results are surprising for such a young system of R&D centres. Using the same source of information for all of them, the Web of Science (WoS), and analysing two main indicators, articles produced between 2012-2014 and Highly cited papers in the same period, we realise that CERCA is the "young brother" of all of them with a minor volume of scientific production but with the highest rate of highly cited papers. A highly cited paper is at the top 1% of the most



cited papers. Therefore they represent the fore front of science for the different disciplines.

Articles	Highly cited papers		%
<b>CNRS</b>	101.380	2.461	2,43%
<b>CSIC</b>	32.486	1.037	3.19%
<b>Max Planck</b>	30.032	1.342	4.47%
<b>CERCA</b>	<b>20.263</b>	<b>1.116</b>	<b>5.51%</b>

*Data Source: Web of Science-Thomson Reuters calculated by BAC  
(<http://bac.fundaciorecerca.cat/>)*

CERCA has managed to find a place in the European scenario based not in quantity but in the quality and the number of citations of the papers. Then, the impact of CERCA scientific production is high absolutely comparable to that of Max Planck, CSIC and CNRS. The table doesn't show the number of researchers of each group of centres. It must be said that CERCA is the smallest among all other institutions of R&D centres.

In funding figures, nowadays, the aggregated annual budget of all CERCA centres is about 400 Million Euros. The contribution of the Catalan Government to the budget is about 30%. The rest of income accounts from competitive projects (mainly EU), private contracts, philanthropy, etc. The CERCA centres have expanded their activity from 2011 to 2014, when an increase in 30 million Euros has been reported in competitive projects. Another very important income in kind at the CERCA centres comes from Universities, Hospitals and other institutions. This part includes the pay-role of many researchers who are at the same time lecturers at University or Doctors in Hospitals. These salaries are mainly paid by the origin institution although they undertake research at CERCA centres, usually located in University campuses or near a Hospital.

In the case of research centres, assessment is a fundamental practice, habitual in the world's most advanced and renowned systems. International standards are well defined in this respect and assessments are usually carried out by an independent panel of renowned experts.

Following a mandate from the Catalan Parliament and, subsequently, from the Government, the CERCA Institution has coordinated the assessment of CERCA research centres with the aim of analysing fulfilment of each one's mission in the last three years. As a result of this action, undertaken between February 2012 and December 2013, more than 500 high added-value recommendations were made to be applied mainly by the management of each centre in the next four years, before the next assessment.

The process of assessment is external and international. In 2012-2013 it involved a total of 202 experts from all over, less than half of whom were from Spanish institutions. The external scientific advisory board of each was strongly involved with the external assessment committees and included researchers from the United States of America (25), Germany (18), the United Kingdom (14), France (11), Italy (9) and a long et cetera that includes the Netherlands, Canada, Sweden, Switzerland, Israel, Korea and Uruguay.

The CERCA assessors came from institutions such as the French National Institute for Agricultural Research (INRA), Institute of Health and Medical Research (INSERM) and National Centre for Scientific Research (CNRS); the German Max Planck Society, Huawei, Fraunhofer Institute and Archaeological Institute (DAI); European institutions such as the European Molecular Biology Laboratory (EMBL), the CERN, the European Space Agency (ESA) and Fusion for Energy (F4E); the University of Tel Aviv; Telecom Itàlia; ALBA Synchrotron, the Spanish National Cancer Research Centre (CNIO), Tecnalia; Swiss Federal Institute of Technology in Lausanne (EPFL); Roslin Institute, Imperial College London, the University of Oxford; Sloan-Kettering Institute, New York, the Bill and Melinda Gates Foundation, Massachusetts Institute of Technology (MIT) and Harvard, Stanford, Princeton and California universities.

In the CERCA evaluation the main criteria are related to the centre's accomplishment of its mission. In cases where the centre's mission is not clearly defined or is expressed in obviously possibilist terms, the mission is established by the Law on fiscal and financial measures of July 2011, which specifically states that: 'Research centres in Catalonia identified as CERCA centres must be entities with their own legal status, non-profit-making, and with headquarters in Catalonia, which have as their main objective research at the frontier of knowledge'.

The Assessment Committee evaluates the accomplishment of the mission trying to answer the following questions:

- What is the quality of scientific production on an international level?
- Is the management structure efficient and proportional to the scientific activity of the centre?
- Is its future vision valid? What infrastructure and resources are required to continue to work in the future?
- Does the centre fit into the Catalan science and technology system, or does it represent duplicity with other structures?
- Are the centres scientific programmes relevant?

As a measure of excellence in European top-research, the CERCA centres have been awarded with 115 ERC grants in the different calls (Starting, Consolidator, Advanced, Prove of Concept and Synergy). 65% centres are

coordinating H2020 projects. In addition 26 CERCA centres have obtained the recognition HRS4R from EU-Euraxess.

In 2012 only 34 ERC grants had been awarded to CERCA centres researchers. Therefore the progress is relevant. The current 115 awards are distributed between 43 ERC starting grants, 30 ERC advanced grants, 17 ERC consolidator grants, 2 ERC Synergy and, finally, 23 ERC Proof of Concept (PoC) grants.

The investment of public funds into CERCA institutes has the permanent condition of accountability. To achieve that objective the CERCA institution is always trying to drive the R&D centres to the right direction, promoting actions such as the recognition and implementation of the Human Resources Strategy for researchers (HRS4R) of the EU Euraxess. In 2014, regarding HRS4R the CERCA institution promoted that CERCA centres could submit applications to obtain the logo for recognition. Up to date already 26 institutes have been recognised with HRS4R logo as can be read in Euraxess webpage. The rest of the CERCA institutes are working hard to obtain the logo and some of them have already made the application and they're waiting for the result of the assessment.

This action shows that CERCA centres are target potential nodes for mobility of researchers in the EU. Recruitment is developed by Open, Transparent and Merit based rules and gender issues among many others are covered and respected by the human resources units in most of the centres.

Accountability could be measured in many different ways. Another one is how CERCA institutes contribute to the economy of the country. In more concrete terms, we assume that the scientific activity sometimes produces results that have a big value. The transference of these results through technology licencing, creation of spin-offs, policy advice, etc. is a clear proof of accountability since society somehow gets back a feed-back from R&D institutions. This is also a priority for CERCA institutes. Up to date over 80 spin off companies have been created by these centres and they are beginning to deal with the secrets of commercialisation of scientific and technological results.

Strategically CERCA centres are preparing their structures for the new European Innovation Council calls that in 2018 will allocate significant funding for innovation among companies and R&D institutions. This is a big challenge not solved at the moment. But initial evidences are encouraging directors and managers to achieve better results in the next years.

The CERCA institution has very recently contributed launching a new Fund for patents named Ginjol. This new tool will support CERCA centres to complement funding for patents applications, valorisation, etc.

## 2. CONCLUSION

The CERCA ex President, Prof. Andreu Mas-Colell, expressed with these words what can be understood as conclusion for this paper:

*“A clear vision rigorously implemented without extreme resources allocation and in the short term drives to the creation of R&D centres able to be competitive at national and international level”*

There are still some challenges pending in the way to success, but in the meanwhile.... This geographic scientific miracle has been catalysed by the CERCA centres. Now Catalan Research is in the map of EU.

(appendix 1) The CERCA centres are:

1. Agrotecnio – Centre for Research in Agrotechnology
2. CED – Centre for Demographic Studies
3. CIMNE – International Center for Numerical Methods in Engineering
4. CMR[B] – Centre of Regenerative Medicine in Barcelona
5. CRAG – Centre for Research in Agricultural Genomics
6. CREAF – Centre for Ecological Research and Forestry Applications
7. CREI – Centre for Research in International Economics
8. CRG – Centre for Genomic Regulation
9. CRM – Centre for Mathematical Research
10. CTFC – Forest Sciences Centre of Catalonia
11. CTTC – Telecommunications Technological Center of Catalonia
12. CVC – Computer Vision Center
13. i2CAT – Internet and Digital Innovation in Catalonia
14. IBEC – Institute for Bioengineering of Catalonia
15. ICAC – Catalan Institute of Classical Archaeology
16. ICCC – Catalan Institute of Cardiovascular Sciences
17. ICFO – Institute of Photonic Sciences
18. ICIQ – Institute of Chemical Research of Catalonia
19. ICN2 – Catalan Institute of Nanoscience and Nanotechnology
20. ICP – Catalan Institute of Palaeontology Miquel Crusafont
21. ICRA – Catalan Institute for Water Research
22. ICRPC – Catalan Institute for Cultural Heritage Research
23. IDIBAPS – August Pi i Sunyer Biomedical Research Institute
24. IDIBELL – Bellvitge Biomedical Research Institute
25. IDIBGI – Girona Biomedical Research Institute
26. IEEC – Institute of Space Studies of Catalonia
27. IFAE – Institute for High Energy Physics

28. IGTP – Health Sciences Research Institute of the Germans Trias i Pujol Foundation
29. IISPV – Pere Virgili Health Research Institute
30. IJC – Josep Carreras Leukemia Research Institute
31. IMIM – Hospital del Mar Medical Research Institute
32. IPHES – Catalan Institute for Human Palaeoecology and Social Evolution
33. IR-Sant Pau – Sant Pau Institute of Biomedical Research
34. IRB Barcelona – Institute for Research in Biomedicine
35. IRB Lleida – Biomedical Research Institute of Lleida
36. IREC – Catalonia Institute for Energy Research
37. IrsiCaixa – Institute for AIDS Research
38. IRTA – Institute of Agrifood Research and Technology
39. IS GLOBAL – Barcelona Centre for International Health Research
40. VHIO – Vall d'Hebron Institute of Oncology
41. VHIR – Vall d'Hebron Research Institute



## NEW OPPORTUNITIES OF PLANT BIOTECHNOLOGY IN ALBANIA USING NANOMATERIALS

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### ABSTRACT

Plant tissue culture is playing an important role in agriculture, industry, and environment. Some Laboratories of Tissue and Cell Cultures are established in Albania (Biotechnology Department of Natural Sciences Faculty, Tirana University; Agricultural Technology Transfer Centre; Tirana Agricultural University). Elite clones, virus free plants of autochthonous fruit species, aromatic-medicinal species etc. are considered our biotechnological products using different methods: micropropagation, meristem culture, embryo culture, hair culture and somatic embryogenesis. Another objective is the conservation of endemic, rare and endangered plant germplasm. Nowadays with nanomaterials, plant tissue culture can be reoriented. The benefits in Albania will be significant in three areas: (i) to establish connections with agriculture and industry in order to improve the efficiency of *in vitro* cultures using nano-sized silver particles as antimicrobial agents in nutrient media; (ii) the *in vitro* techniques products (clones, virus free plants, hybrids etc.) and improved or new secondary metabolites, that can be exploit in pharmaceutical, cosmetic or other industries (through cell suspension culture) using nanomaterials will be in the future in the Albanian markets; (iii) improvement of crops by transgenesis, in which *in vitro* cultures allow foreign gene transfer through nanoparticles. Special species of Albanian plant resources are excellent candidate for isolation of genes involved in the drought and other stress tolerance mechanisms; (iv) use of *in vitro* callus and hair culture to produce plant derived silver nanoparticles.

**Keywords:** plant micropropagation, nutrient media, nanotechnology, nanomaterials

### 1. INTRODUCTION

Plant tissue culture is a basic and fundamental component of plant biotechnology, and progress in different fields of biotechnology greatly depends on the improvement of this technique. Nowadays, micropropagation is a suitable method for obtaining a large quantity of genetically homogeneous and healthy plant material which can be used for planting (Kongjika *et al.*, 2002, Gamborg, 2002). The rapid *in vitro* multiplication of clonal plants is

desirable to shorten crossing programs in fruit tree breeding (Daorden *et al.*, 2004).

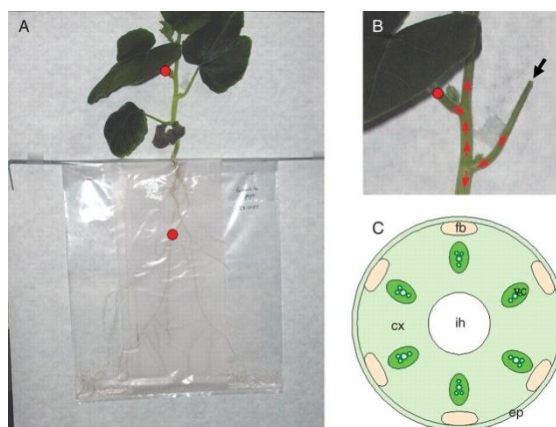
Despite all advantages, modern research has found a useful and very important connection between plant *in vitro* techniques and nanotechnology. The field of nanotechnology is one of the most active areas of research in modern materials science. There might be several possible applications of nanotechnology in agriculture starting from crop production, fertilizer and irrigation management, crop protection and crop improvement for quality and agronomic traits. Nanotechnology-based reorientation of agriculture can boost production of quality food. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Uses of nanoparticles in crop sciences are consistently increasing. Silver nanoparticles have remarkable uses in crop production. Plants grown in nutrient medium provided with nanosilver can uptake and accumulate nanoparticles (Lee *et al.*, 2012). Nowadays, synthesis of silver nanoparticles from plant is evolved into an important branch of nanotechnology (Malabadi *et al.*, 2012). Nanoparticles are used during micropropagation even for increasing the percentage of explants which produce shoots and the shoots number/explants (Aghdai *et al.*, 2012).

During *in vitro* propagation, internal and external contamination of plant tissues turns out to be a prevailing problem, because microorganisms, mostly fungi and bacteria, can grow much faster than plant cells and take up all the nutrients, especially during fruit trees micropropagation (Cassells, 1991; Sota & Kongjika, 2014; Grazhdani *et al.*, 2014). Although there are a variety of techniques to minimize the possibility of bacterial and fungal contaminations during *in vitro* propagation, such as meristem culture (the pathogen-free part of the plant) and repetitive subcultures, it is very important to find other techniques in order to overcome contamination problems. One of the potential applications in which silver can be utilized is in management of plant diseases. For this purpose, the use of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical (Mahna *et al.*, 2013; Sondi *et al.*, 2004).

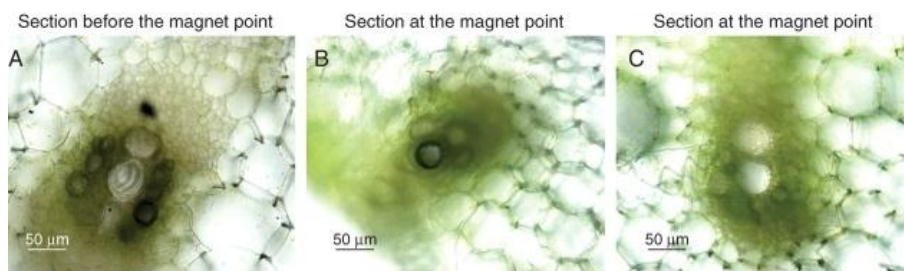
Recently, many authors reported the efficient delivery of DNA and chemicals through silica nanoparticles internalized in plant cells, without the requirement of specialized equipment. Bioferrofluids can be introduced into whole living plants, can travel using the vascular system and can be concentrated in specific areas by application of magnetic gradients. Gonzales *et al.*, 2008 studied the application of carbon-coated magnetic nanoparticles in pumpkin (*Cucurbita pepo*) and their transportation into cells (Fig. 1). The preliminary results of the study showed the presence of nanoparticles both in the extracellular space and within some cells (Fig. 2).



Further work is needed to evaluate how the nanoparticles penetrate and are transported within the plants, and the mechanism(s) of intracellular internalization to explore the potential of nanoparticles as smart treatment-delivery systems in plants.



**Figure 1.** (A) Pumpkin plant growing in the polyethylene bag system. Red circles indicate where magnets were placed. (B) Detail showing the point of application of the bioferrofluid (black arrow) and further expected movement of nanoparticles through the vascular system (red arrows). (C) Schematic representation of a transverse section of the pumpkin stem. ep, Epidermis; cx, cortex; fb, fibres; vc, xylem vessels; ih, internal hollow (Gonzales *et al.*, 2008).



**Figure 2.** Hand-cut sections of petioles (A–C) of pumpkin plants treated with bioferrofluid. (A) Detail of vascular tissues at the application point. Dark coloration indicates accumulation of bioferrofluid. (B) Detail of vascular tissues adjacent to a magnet. Bioferrofluid is concentrated in xylem vessels. (C) Detail of vascular tissues opposite a magnet placement. No bioferrofluid accumulation is observed (Gonzales *et al.*, 2008).

Tissue culture plays an important role for the *in vitro* production of secondary products through callus culture or hairy root culture. Secondary metabolites have a diverse range of properties (pharmacological, medicinal, hemolytic, antimicrobial etc.). The reports show that the use of nanoparticles

increases the production and/or the effect of the secondary products produced by the plants (Ghanati & Bakhtiarian, 2014; Ghasemi *et al.*, 2015).

There are a number of methodologies have been used for a long time in traditional plant transgenic methods such as particle bombardment, ultrasound etc. However, these methods have many limitations and disadvantage as most of the times naked DNA is damaged or destroyed. In this new technology era, researches proved that nanoparticles can avoid DNA damage and enhance transformation effectiveness (Yu-qin *et al.*, 2012; Sokolova *et al.*, 2006).

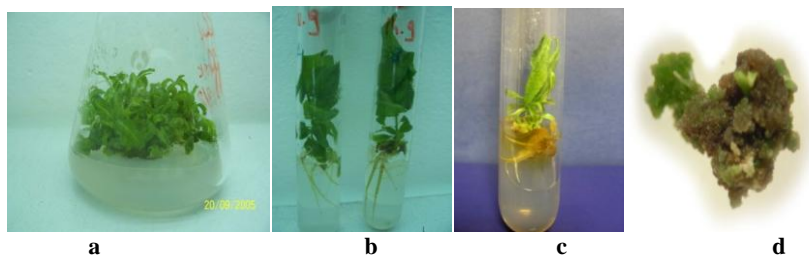
The aim of this study is to represent some of the main areas of micropropagation of autochthonous Albanian plant materials and the possibilities for the use of nanoparticles to overcome some important problems and/or to improve further production and multiplication of plant materials or products.

### **1. Solutions of the problems related with micropropagation of some autochthonous fruit trees under aseptic conditions by nanomaterials use**

Ethnobiological studies conducted in the Western Balkans in recent years have reported rich biocultural diversity and a remarkable vitality of traditional knowledge concerning the local flora in this region (Pieroni *et al.*, 2013). In some Laboratories of Tissue and Cell Cultures, established in Albania (Biotechnology Department of Natural Sciences Faculty, Tirana University; Agricultural Technology Transfer Centre; Tirana Agricultural University) is used the plant tissue culture with an important role in agriculture, industry, and environment.

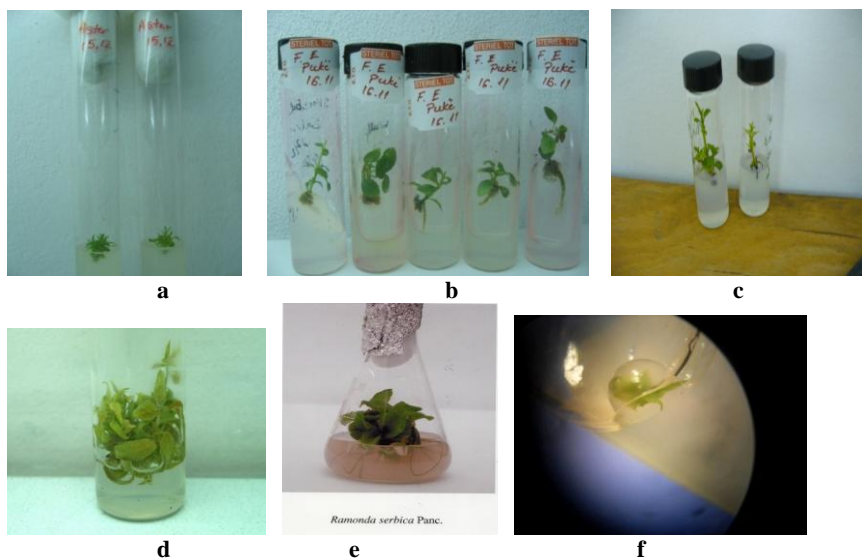
During several years there are conducted a lot of experiments with the purpose of propagation and conservation the important species which originate from autochthons local varieties like wild cherry (*Prunus avium* L.); wild almond (*P. webbii* Spach Vierh.); wild cherry plum (*P. cerasifera* L.); mahaleb or rock cherry (*P. mahaleb* L.); blackthorn berry (*P. spinosa* L.); wild pear (*Pyrus pyraeaster* L.); pomegranate (*Punica granatum* L.); myrtle (*Myrtus communis* L.); wild apple (*Malus sylvestris* L. Mill.), forsythia (*Forsythia europea* Degen. & Bald.), etc.

Elite clones, virus free plants of autochthonous fruit species, aromatic-medicinal species etc. are considered our biotechnological products using different methods: micropropagation (Fig. 3a, sage), meristem culture (Fig. 3b, wild cherries), embryo culture (Fig. 1c, wild almond), hair culture and somatic embryogenesis (Fig. 3d, pomegranate).



**Figure 3.** Some biotechnological products using different *in vitro* culture methods: (a) – sage plantlets produced by micropropagation (Kongjika, E.); (b) – virus-free wild cherry plantlets produced by meristem culture (Kongjika, E.); (c) – rooted wild almond plantlet regenerated by embryo culture (Sota, V.), (d) – new pomegranate buds regenerated from callus by somatic embryogenesis of hair culture (Kongjika, E.).

Another objective is the conservation of the germplasm of endemic, rare, endangered species and species with economic values (Fig. 4a,b,c,d,e,f).



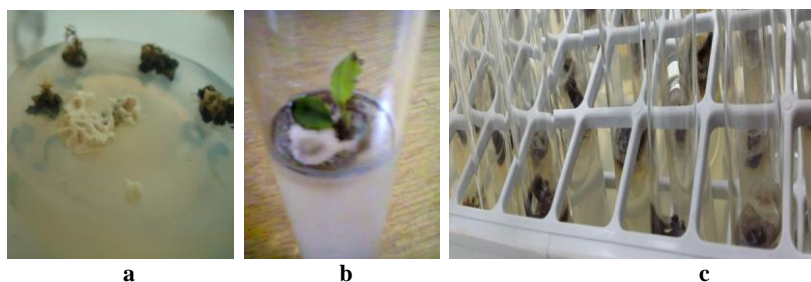
**Figure 4.** Conservation by minimal growth method of germplasm of some endemic, rare, endangered species and species with economic value: (a) – endemic species, *Aster albanicus* subsp. *paparistoi* (Kongjika, E.); (b) – endemic species, *Forsythia europaea* (Kongjika, E.); (c) – aromatic-medicinal species, *Myrtus communis* (Sota, V.); (d) – autochthonous wild cherry *Prunus avium* (Kongjika, E.); (e) – rare Balkanic species, *Ramonda serbica* (Kongjika, E.), and (f) – wild cherry buds preserved by encapsulation-dehydration method (Sota, V.).

Although, advantages of micropropagation, there are still problems associated with its commercial applications. A main problem during *in vitro* technique is a high rate of contamination that led to a high loss of explants

during subcultures. For explants sterilization are used different reagents such as Bavistine 0.2%,  $\text{HgCl}_2$  0.1%, ethanol 70%, NaOCl etc. (Sota., 2012; Grazhdani *et al.*, 2014). Despite the success of these methods in many cases, the resistance of bacteria and fungi to common bactericides and fungicides in a period of time after contamination is a limiting factor in the sterilization of plant and media materials. For this reason, in many cases, the protocols for sterilization results non-effective in disinfecting explants derived from field-grown adult trees. The contamination rates in some cases are about 80% (Grazhdani *et al.*, 2014). It results that even mercuric bichlorid, known more effective in other experiments (Kongjika *et al.*, 2002) does not affect the avoidance of infections. Also, some antimicrobial chemicals have phytotoxic signs, like reduction of explants' growth rate or genetic mutations.

Antibiotics are commonly used in the medium to eliminate unwanted contaminants from plant systems and it resulted successful in overcoming contamination problems during *Prunus* sp. micropropagation (Bode, 2011). However, antibiotics are frequently phytotoxic otherwise may retard or inhibit plant tissue growth. Most antibiotics have been shown inhibitory effects in the plants (Abdi *et al.*, 2008).

The levels of microbial and fungal infections are directly affected by the type of the explants (Fig. 5a,b,c). Buds are the explants that show the highest contamination rates, due to the fact that they are in direct contact with contaminated area (air, soil etc.), but most often shoot tips and meristems are the explants of choice due to their genetic stability. Must be noted that the explants are isolated from the field trees where the contamination is very high and for this reason require the use of specific agents with antimicrobial and/or antifungal effect.

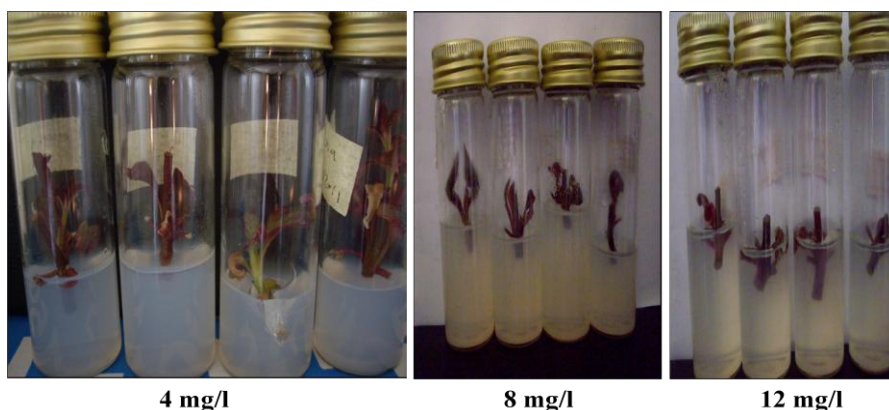


**Figure 5.** Contamination of different explants of fruit trees species during *in vitro* culture: (a) – calli with new pomegranate plantlets (Kongjika, E.); (b) – buds of wild apple (Grazhdani, M.), and (c) – forsythia buds (Kaçorri, A.)

New applications of nanoparticles and nanomaterials are emerging rapidly because to their antimicrobial and antifungal effect. Some reports have shown that antimicrobial components in the form of nanoparticles could be used as

bactericidal materials in the plant tissue culture. Silver-based compounds such as silver nitrate have long been recognized as highly toxic to microorganisms, and show strong antibacterial activity on broad strains of bacteria. The detrimental effects of nano silvers have been shown more than 600 microorganisms (Sondi & Salopek-Sondi, 2004; Fakhrfeshani *et al.*, 2012; Mahna *et al.*, 2013). In other reports has been investigated extensively the effect of titanium dioxide nanoparticles  $\text{TiO}_2$  for the killing or growth inhibition of bacteria (Safavi, 2014).

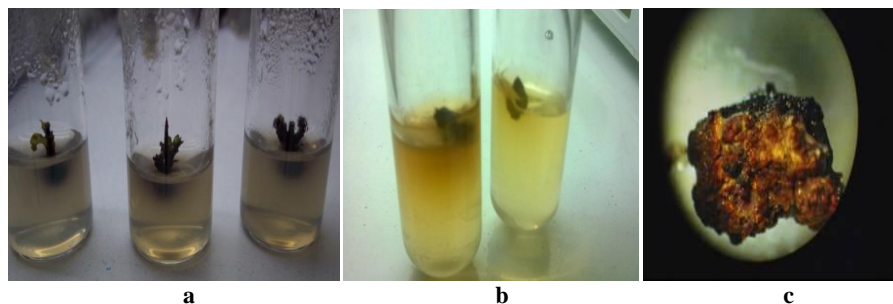
Nabeel,(2011) conducted a research using explants cultured in MS medium supplemented with different concentrations of silver nano particles 4, 8, 12 mg/l. to assess the efficiency of using these particles in control the infection percent. The results indicate that healthy plants percent were increase from 45% to 100% in all treatments. However, in 4 mg/l treatment the explants were continued to grow better even after 28 days in culture (Fig. 6).



**Figure 6.** Different growth of explants in MS medium with 4, 8,12 mg/l silver nanoparticles after 28 days in culture (Nabeel, 2011)

Apart bacterial or fungal contamination, a major problem, especially when propagating fruit trees is the polyphenolic oxidation during tissue culture which is observed by the browning of nutrient medium despite the presence of antioxidants (ascorbic acid) in it, or the experiments conditions (dark treatment during the first phase of inoculation for 24 hours and frequent transfer of explants to fresh medium) (Fig. 7a,b,c).





**Figure 7.** Poliphenolic oxidation of: (a) - *Punica granatum*; (b) - *Malus domestica* explants;  
(c) – *Quercus ilex* explants during *in vitro* culture

This problem appears when using different explants such as: (i) mature zygotic embryos of *Juglans regia* (Zekaj *et al.*, 2000) *Prunus webbii* (Sota, 2012) or (ii) buds as primary explants in *Punica granatum* (Kongjika *et al.*, 2014) (Fig. 7a), *Malus sylvestris*, *Malus domestica* (Grazhdani *et al.*, 2014) (Fig. 7b), *Prunus avium* and *Prunus mahaleb* (Sota, 2012), *Quercus ilex* (Kongjika *et al.*, 2002) (Fig.7c), which exhibit browning or yellowing at the cut ends soon after their excision and this subsequently deters the growth of the explants and this phenomenon subsequently leads to the death of the explants.

The preparation of explants for establishment under *in vitro* conditions exposes the polyphenolic compounds to copper containing enzymes polyphenol oxidases. The polyphenolic compounds on oxidation with these enzymes form quinines which are highly reactive and non-specifically polymerize proteins and produce dark pigments, melanin.

Several authors (Wang *et al.*, 1994; Modgil *et al.*, 1999) used several antioxidants such as ascorbic acid, citric acid, cysteine either alone or in different combinations with other adsorbing agents for the intitation of cultures of an apple cultivar. But, in many cases, even these methods results ineffective in order to overcome this problem.

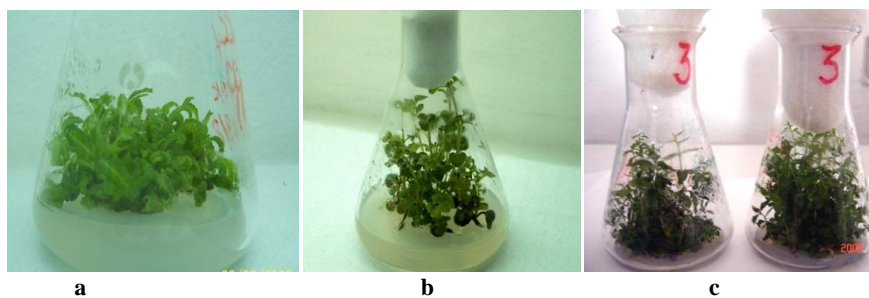
The effectiveness of nano silver particles as an antioxidant and polyphenol absorbent compared to silver nitrate and activated charcoal on different explants is reported by several authors. When using silver nanoparticles, no browning was observed during surface sterilization and the culture period of *Rosmarinus officinalis* L. (Nartop, 2016), of *Hevea brasiliensis* (Moradpour *et al.*, 2016). Other reports have determined that the use of TiO<sub>2</sub> nanoparticles during micropropagation of *Cicer arietinum* L., resulted in decreased levels of H<sub>2</sub>O<sub>2</sub> and the antioxidant enzymes were more effective in cell protection (Mohammadi *et al.*, 2014).

Nano-agriculture involves the employment of nanoparticles in agriculture with the ambition that these particles will impart some beneficial effects to the

crops. These particles can serve as effective nano-transporters to deliver DNA and small dye molecules into intact plant cells.

## 2. Micropropagation of medicinal plants and future possibilities for secondary metabolites production using nanomaterials

Mediterranean Albania is rich in different aromatic-medicinal plant species such as sage (*Salvia officinalis*), oregano (*Origanum. vulgare*), myrtle (*Myrtus communis*) etc. Between them, sage has been the most exploited during the last decade, endangered also from deforestations, fires, drought, soil erosion, presence of new infrastructure in the forests etc. This spontaneous species is classified as endangered according the “Albanian Red Book” (Vangjeli *et al.*, 1995). About 80% of the world production of sage is harvested from Albanian and Montenegrin areas (exported in USA and Europe) (Asllani, 2000; 2002). The sustainable use of medicinal plants can be achieved better through their cultivation (Fig. 8a,b,c).



**Figure 8.** *In vitro* propagated plantlets of: (a) – sage (Kongjika, E.); (b) – oregano (Kongjika, E.), and (c) – myrtle (Sota, V.)

Plants are requisite source for the production of chemical substances like secondary metabolites and phytochemicals which find relevance in the pharmaceutical, food and flavour industries. Preliminary genotype selection could be of high importance for developing programs of genetic improvement in order to obtain biotypes with agro-industrial and environmental potential. DNA-based molecular markers have been used in a wide range of plant species either for cultivar identification or in assessment of genetic relationships between individuals and species. A number of researches (Bacu *et al.*, 2008; Bacu *et al.*, 2011; Lakuriqi *et al.*, 2011) are undertaken in Albania on the evaluation of biodiversity in molecular level at local resources of some aromatic plant species.

In 1988, when Chiang Mai Guidelines on the Conservation of Medicinal Plants were drafted new technologies such as micropropagation and *in vitro* preservation had only just appeared on the horizon of possibilities for *ex situ* production and preservation of plant germplasm. The sage plants obtained

through micropropagation had higher concentrations of essential oils comparing to plants cultivated *ex vitro* (Ioja-Boldura *et al.*, 2010; Ruffoni *et al.*, 2009; Gounaris *et al.*, 2002).

Nanoparticles can be appropriate candidates for elicitation studies of *in vitro* secondary metabolites production. It resulted that zinc and iron nano-oxides were suitable for the accumulation of hypericin and hyperforin in cell suspension culture of *Hypericum perforatum* (Sharafi *et al.*, 2013). Another studied presented the positive effect of nanoparticles on callus culture development and production of natural antioxidants in *Phaseolus vulgaris* (Fazal *et al.*, 2016).

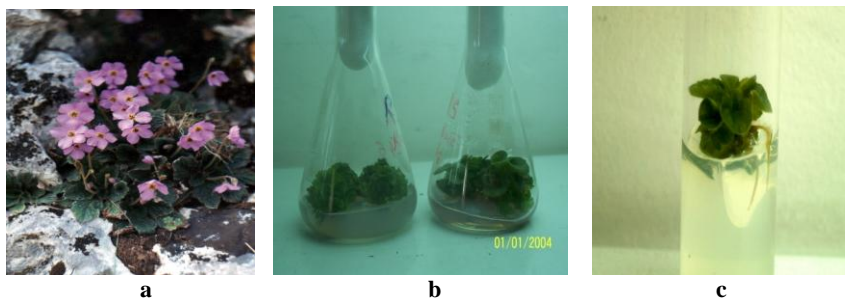
The successful application of the nanoparticles offers as a new strategy for improving secondary metabolite production. Future research is needed to fully elucidate the effects of nanoparticles on production mechanisms of secondary metabolites in medicinal plants.

### **3. Improvement of crops by transgenesis, in which *in vitro* cultures allow foreign gene transfer through nanoparticles**

Since the late 80s, transgenesis by particle bombardment has become the important method for the production of transgenic plants with improved characteristics, especially crops tolerant to biotic and abiotic stresses, very present last decades in agriculture. Some crops like rice, maize and other grasses are produced by this method for the introduction of various genes. In this method, large numbers of DNA-coated, micron-sized metal (gold or tungsten) particles are shot at target cells or tissues using a biolistic gene gun. With the availability of modern biotechnological methods of protoplast culture and regeneration systems for several plant species, the development of new nanodevices and nanomaterials opens up new opportunities for novel applications in agricultural biotechnology (Nandakumar *et al.*, 2011).

Special species of Albanian plant resources are excellent candidate for isolation of genes involved in the drought and other stress tolerance mechanisms. Due to their characteristic of "resurrection plants" (Gashi *et al.*, 2013), ramonda plants can survive in different climatic conditions. The extreme drought tolerance and the geographic isolation make this species the excellent candidates for isolation of genes involved in the mechanism of drought resistance. In future, the selected cDNAs will be used for producing of modified crops to improve crop resistance to stress (Minkov, 2009). The elaboration of the tissue culture of this "resurrection species" in our laboratory (Fig. 9a,b,c) (Kongjika *et al.*, 2002) and the genetic transformation system of these plants transmitted by *Agrobacterium tumefaciens* or by nanodevices in the future will open the opportunity for the production of transgenic plants with tolerance to abiotic and biotic stresses.





**Figure 9.** (a) - *Ramonda serbica* plants of Petrela region (Albania) (Kongjika, E.); (b) - *Ramonda serbica* plantlets (Kruja) in bundles, ready to the subculture (Gashi, B.) and (c) - Rooted plantlets of *Ramonda serbica* (Kongjika, E.)

#### 4. Use of *in vitro* callus and hair culture to produce plant derived silver nanoparticles

The silver nanoparticles have potential applications in the biomedical field. This simple procedure has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as large scale commercial production. Therefore, synthesis of silver nanoparticles from plant is evolved into an important branch of nanotechnology. Production of silver nanoparticles using plants provides advancement over traditional chemical and physical methods as it is cost effective, easily scaled up for large scale, and no toxic chemicals were used during synthesis (Malabadi *et al.*, 2014). Modern green nanotechnology is considered very safe because of the use of non-toxic chemicals and simple solvent water for synthesizing nanoparticles. This nanotechnology facilitates also the production of smaller silver nanoparticles with low toxicity to human and greater efficacy against bacteria. In general silver ions can bind with a variety of negatively charged molecules like RNA, DNA and proteins. The antibacterial property of silver nanoparticles increases because of their larger total surface area per unit volume.

Furthermore, these green methods are very low cost, fast, and efficient and generally lead to the formation of crystalline nanoparticles with a variety of shapes (Kouvaris *et al.*, 2012). For example, it is concluded that the biosynthesized silver nanoparticles of callus and root aqueous extract of *in vitro* derived medicinal plants of *Clitoria ternatea* provided potential toxicity against tested clinical samples which could be used for prevention of several deadly diseases. In another study, silver nanoparticles were synthesized from aqueous silver nitrate through a simple and eco-friendly route using leaf broth of *Arbutus unedo* (a common Albanian species), which acted as a reductant and stabilizer simultaneously. The aqueous silver ions when exposed to the leaf broth were reduced and stabilized over long periods of time resulting in the green synthesis of surface functionalized silver nanoparticles.

In the future, the green nanotechnology has great possibilities for application in Albanian plant genetic resources, which are valuable sources of medicinal and pharmaceutical components.

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## DEVELOPMENT OF ELECTROCHEMICAL SENSORS AND BIOSENSORS USING NANOMATERIALS AT THE UNIVERSITY OF TIRANA, ALBANIA

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University of Tirana, established in 1957, is an education and research institution offering 180 study programs in 6 faculties with more than 30.000 students and 1100 academic staff. The Faculty of Natural Sciences (FNS) as part of UT is the main centre in Albania for training specialists and conducting research in the fields of Mathematics, Computer Sciences, Physics, Chemistry, Biology and Biotechnology. Recently knowledge on nanomaterials, electrochemical (bio)sensor are introduced in the curricula of several disciplines. FNS through publications in scientific journals it is confirmed as a centre in which the scientific studs are orientated in the field of nanomaterials, biosensor and their applications. The most experienced groups work using optical and electrochemical methods, developing electrochemical sensors and biosensors based on surface modifications, composite materials involving electrochemical techniques. Albnano related activities consist on: (i) chemoresistors for Hg detection (100 nm width; 42 nm thickness), thinner gold layer results in higher sensitivity. The system for calibration of sensors for mercury vapour is developed (ii) carbon nanotube (CNT) epoxy composite electrode are used for antibiotic determination. (iii) modified screen-printed electrodes for determination of amino acids; histamine and heavy metals are developed. (iv) Home- made sensor (CPE), modified with enzyme, plant tissue, nanomaterials are used for determination of phenolic compound and heavy metals.

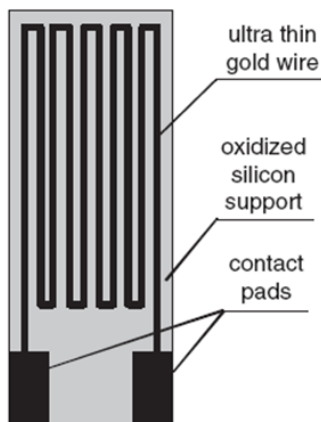
**Key word:** affinity sensor, calibration, thermoinjection, modified sensor, composite, SPE, CPE

### **Affinity sensors for Hg vapor**

Although the first chemical sensor for detection of mercury vapour was suggested more than 40 years ago, there is still a high potential for its further development. The main directions of this progress will probably include a design of new selective filters, an integration of calibration system into sensor devices, and an evaluation of new detection principles. The requirement to develop inexpensive but highly sensitive and highly selective sensor for mercury vapour was a motivation to apply non-traditional analytical approaches based on the concept of chemical affinity sensors. This approach was realized by using a thin gold layer as the receptor. Mercury adsorption

onto this receptor was indicated by decreasing electrical conductance of thin gold layer. However, this effect is not selective: similar changes are caused by adsorption of water vapor, sulfuric compounds. etc.

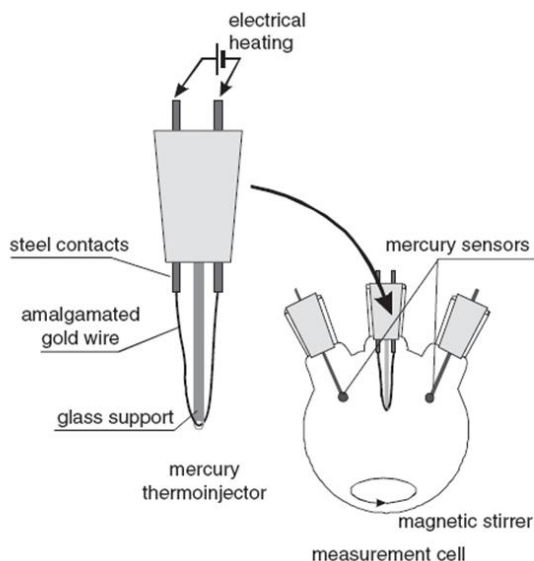
A thin gold layer as the receptor for Hg has a meander shape of 100 nm width and 42 nm thickness as it is shown schematically in Fig.1. The gold electrode is coated by a monolayer of 1-hexadecanethiol detailed description in Mirsky et al (2002).



**Fig.1** Configuration of the chemical sensors for detection of mercury vapor. Mirsky et al (2002).

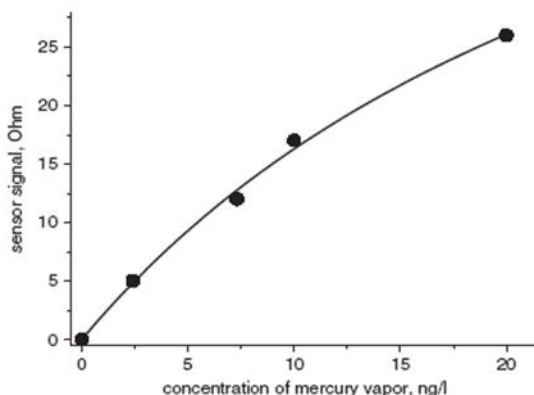
Sensor calibration is performed by thermoinjection of nanogram-amount of mercury quantitatively deposited by electrochemical reduction. Gold wire electrodes with diameter of 50 or 100 nm were used (Vasjari, 2000). Metallic mercury was formed on the gold wires by electrochemical reduction from water-soluble mercury salt. Thermoinjection is performed by heating of the wire electrode placed in mercury measurement chamber (Fig 2). Resistance of sensors placed simultaneously in the calibration flask are measured at dc or ac mode with sensitivity at least 0.1%.





**Fig.2** The system for calibration of sensors for mercury vapor. (Vasjari, 2000)

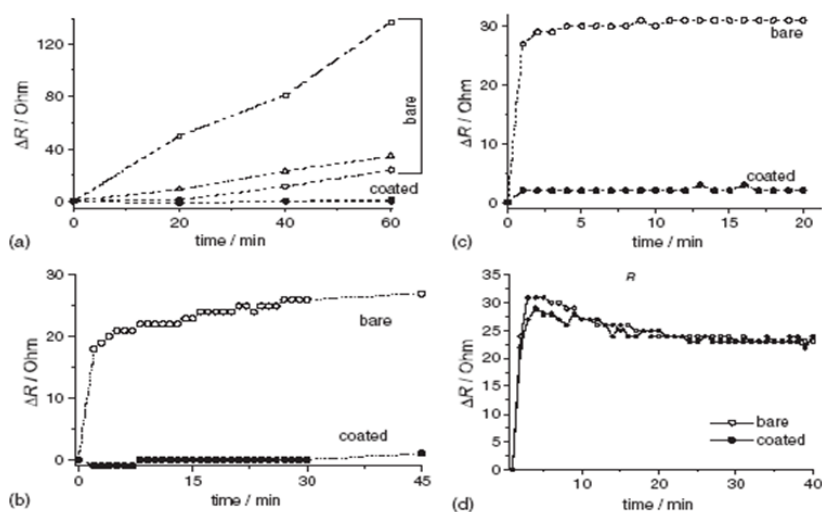
The concentration of mercury vapor is determined from the calibration curve. Typical calibration curve is shown in Fig.3. Detection limit corresponds to about 1 ng/l. Sensor regeneration was performed by heating at 150°C for 10 min.



**Fig. 3** Typical calibration curve (Vasjari, 2000)

A perspective solution to avoid interferences is the coating of gold by self-assembled monolayers of alkylthiols. In 1987, a blocking effect of self-assembled monolayers of alkanethiols on Faraday's processes was described. This was a strong motivation for intensive investigation of this system.

Improvement of selectivities was achieved by using the monomolecular layer of alkylthiols as a filter for several interferents. While conductometric responses of coated and uncoated gold layers on exposure to mercury vapor are similar, a strong difference in their responses to other compounds is observed (Fig.4). No response of the coated sensors to saturated water vapor was detected, practically no effect of either vapor of sulphuric acid, or butanethiol, was observed on coated sensor.



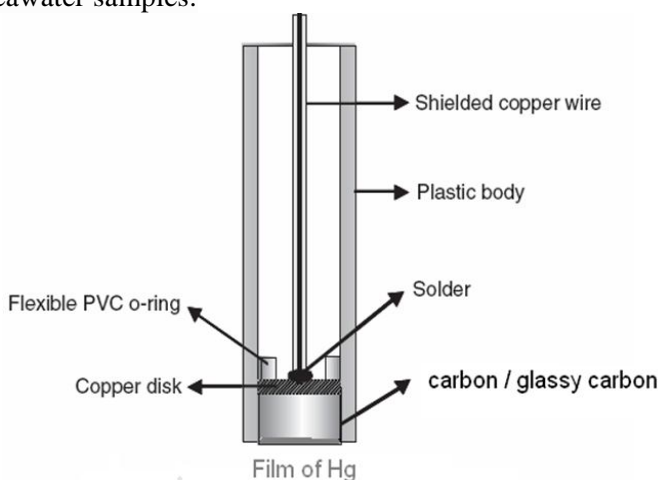
**Fig.4** Influence of typical interferents on mercury sensors based on changes of lateral resistance of thin gold layers. Sensors: bare gold layers (open symbols), gold layers coated by self-assembled monolayers of 1-hexadecanethiol (filled symbols). Interferents: 100% humidity (a), saturated vapour of sulphuric acid (b), 10 mg/l of 1-butaneethiol (c), 10 mg/l of iodine vapor. Thickness of gold layers: 42nm (Mirsky et al, 2002)

The observed selective permeability of alkanethiol monolayers can be caused by different factors mainly related with the polarity of the molecules as are explained in the original paper (Mirsky et al, 2002). The finding that mercury vapor can penetrate a monolayer of alkanethiols on gold, while most of interferents cannot, allows one to design highly selective sensors for mercury vapor without additional macroscopic pre-treatment modules. Thus, a simple integrated analytical system including a filter and a sensor, both scaled down almost until the technical limit (monomolecular layer as the filter and tens or hundreds of atomic layers as the sensor) can be prepared.

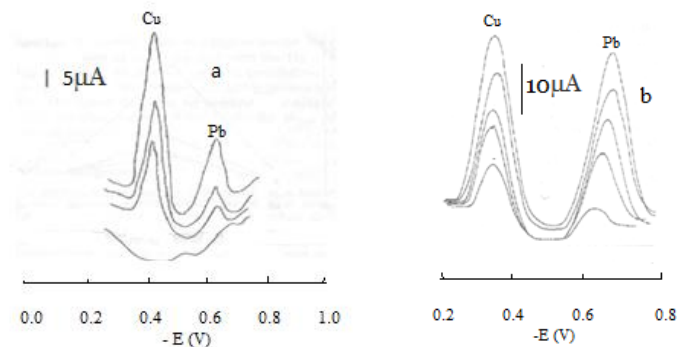
### Sensors based on surface modifications for heavy metals

Traditional analytical methods for determination of heavy metals (HM), are extremely sensitive but there are relatively expensive and require

analytical pre-treatment procedures. Therefore, in some cases, it is required to find alternative ways and replace them by more easy-to-use and inexpensive ones such as electrochemical transduction based on sensor development. Most sensors for determination of HM have been developed using electrochemical transducers because of the simplicity of construction and cost. Using different types of modifiers and modification procedures the degree of selectivity and sensitivity may be introduced into the sensor response. By modifying the surface of a glassy carbon electrode the determination of lead and copper simultaneously is studied. Two different modifying ways are studied and compared: i) the surface of glassy carbon electrode was coated with a thin Hg film by electrodeposition process using strictly experimental conditions. The optimization of deposition potential, deposition time and the concentration of Hg(II) in the electrolytic cell was required to prepare the best thin film of Hg deposited onto the surface of glassy carbon electrode by which will define stripping peaks of Cu and Pb were obtained. The other modification approach was by coated mechanically the surface of GCE using a thin film of Hg:Nafion as a modifier. The mixture of Hg(II) chloride solution with a nafion solution diluted in ethanol is used to coat the polished glassy carbon surface according to the procedure described in Merkoci et al., (2000). In this case only the amount of the modifying solution was required to optimize. The optimized film was used to determine Pb and Cu simultaneously based on well-defined stripping peaks (Fig.6). The sensor is shown schematically in Fig 5. The type of inert supporting electrolyte, the pH as well as the experimental condition of ASV was found to play an important role on the sensitivity of the measurement in both cases. The modified working electrodes were used to determine Pb and Cu in real seawater samples.



**Fig 5.** Configuration of the Glassy Carbon Electrode modified with Hg film



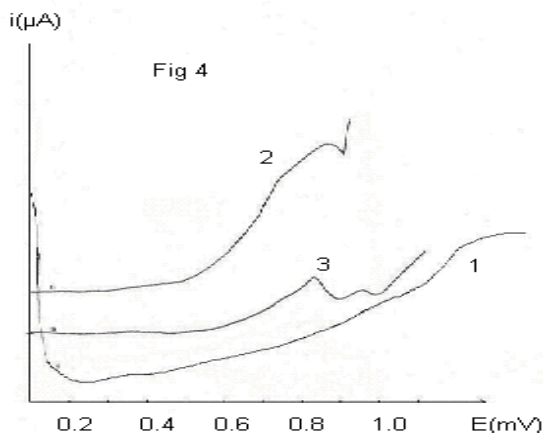
**Fig 6.** Typical stripping curves for Pb and Cu; a) electrochemical modification with Hg film b) mechanically modification with Hg-nafion film (Merkoci et al, 2000)

Results for Pb and Cu determinations show the advantages of Hg-nafion modification of the glassy carbon electrode. These advantages include a shorter modification time, the avoidance of the manipulations with Hg solutions during the formation of the Hg film and also improvement of sensitivity for metals determination was noticed. The modification of traditional working electrodes based on mechanical procedures open the new possibilities for the design of single-use devices using screen-printing procedures.

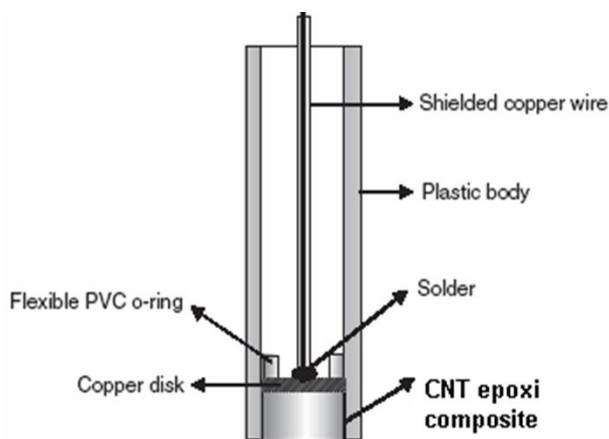
### Composite sensors based on CNT modification

The field of chemically modified electrodes (CMEs) has received considerable attention because of the possibilities for more direct control over the chemical nature of an electrode and are able to accumulate the analyt based on the interaction with a functional group on the electrode surface. They also provide a renewable and modified surface, are inexpensive, and generate very low background current interferences. The electronic, chemical, and mechanical properties of nanomaterials, such as carbon nanotubes (CNT), make them extremely attractive for electrochemical sensors in comparison to conventional materials. The extremely high surface-to-volume ratios associated with these nanostructures make the electrical properties extremely sensitive to species adsorbed on surfaces, leading to an increased mass-transport rate and fast electron transfer, providing excellent sensitivity and selectivity. The CNT epoxy-composite was prepared by dispersion of multi-wall CNT inside the epoxy resin for antibiotic determination (Fig. 8). The NTC modified CPE was compared with unmodified CPE based on their analytical performance when the analyte was one of the antibiotics (gentamicine or amoxicillin). The shapes of voltammograms obtained

using the modified und unmodified composite sensors (Fig.7) obviously show the improvement of the shape of the voltammogramme with define analytical peak. Also the advantages of CNT based electrodes are relating with the oxidation potentials, sensitivities as well as the stability of the responses.



**Fig 7.** Voltammogrames obtained in buffer solution (1); Amoxicilin solution 0,049 mg/ml using: unmodified CPE (2); NTC modified CPE (3)



**Fig 8.** Configuration of the composite electrode

## Screen-printed electrodes for determination of amino acids; histamine and heavy metals

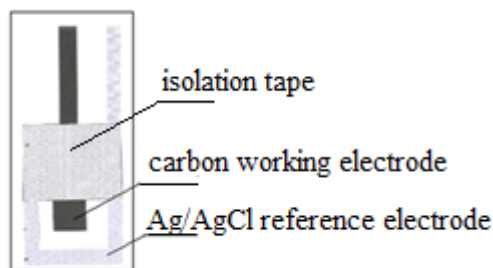
### i) Screen-printed electrodes for determination of amino acids

Various electroanalytical methods and sensors for the detection of aminoacids have been reported using conducting electroactive polymer modified microelectrodes, hanging mercury drop electrode in the presence of

nickel ion, copper microelectrode, modified glassy carbon electrode, disposable gold working electrodes, a platinum electrode, a multiwall carbon nanotube (MWNT) film modified electrode, etc.

Screen-printed electrodes represent one of the most interesting alternatives in the design of electrochemical sensors. The use of these electrodes have the advantage of disposability, so after one use they are discarded avoiding the problems related with the memory of the previous measurements. In this work, it was described the use of bare screen-printed carbon electrodes, in a two-electrode system for determination of aminoacids.

Arrangement (Fig 9) consisting of an unmodified carbon working electrode and an Ag/AgCl/Cl<sup>-</sup> reference electrode printed in a single strip. To highlight the advantages of SPE, the experiments have been conducted using other types of working electrodes: graphite/ platinum disc electrodes using linear sweep voltammetry in the same experimental parameters (LSV). These electrodes were also applied to real samples to demonstrate the possibility for simple and faster analysis of pharmaceutical samples (Vasjari et al, 2005).

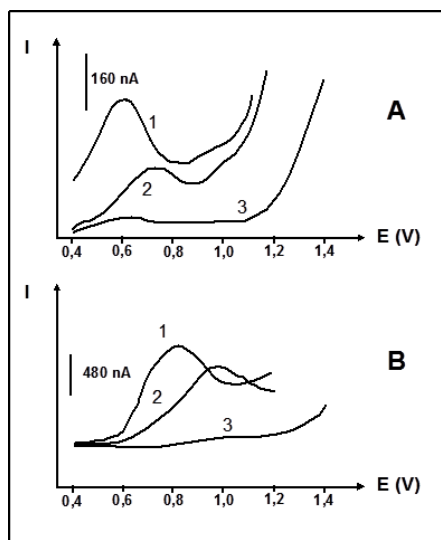


**Fig 9.** Configuration of the SPE; working electrode and reference electrode printed in the same strip.

Firstly the oxidation potential of the analytes (cysteine and tyrosine) using for each of the electrodes was found. The operating potential should be high enough to drive the redox reaction of interest, but low enough to avoid interfering signals due to oxidation of other biomolecules present in a sample. The corresponding LSV parameters for the three electrodes and both amino acids are summarized in Table 1. As Fig. 10 shows, the LSV curves for the SPES represent well-defined oxidation peaks at lower potentials compared to those recorded at carbon and platinum disc electrodes.

**Table 1.** LSV parameters determined for tyrosine and cysteine measured at various electrodes (Vasjari et al, 2005)

Working electrode	Oxidation potential E (V)		Anodic current density I (nAmm <sup>-2</sup> )	
	Tyrosine	Cysteine	Tyrosine	Cysteine
Platinum electrode	0.9	0.61	30	101
Carbon electrode	0.94	0.71	12	8
SPES	0.77	0.55	178	57



**Fig.10.** Linear sweep voltammetry responses of cysteine (A) and tyrosine (B) for SPES (1), carbon (2) and platinum (3) electrodes. The concentration of each amino acid was  $2.4 \times 10^{-3}$  M. The measuring solution was 0.1M phosphate buffer pH=7.0 and 0.1M KCl. (Vasjari et al, 2005)

Next experiments were calibration studies on Cysteine and Tyrosine by Amperometry in stirred Solution. Figure 11 indicates typical calibration plots obtained for cysteine and tyrosine in 0.1M phosphate buffer solution, pH 7. The figure shows three replicate studies using the same SPES. Obviously the sensitivity of the SPES was greatest for the first measurements and then declined in the subsequent measurements. From this study it was concluded that electrode fouling occurred and could be eliminated by using one SPE for each measurement in a calibration run as well as for each sample. The reproducibility of the SPES responses was considered very important to investigate. Series of eight successive measurements for cysteine and six for tyrosine, each recorded on a new SPES, yielded RSDs of 4.6 and 4.1%,

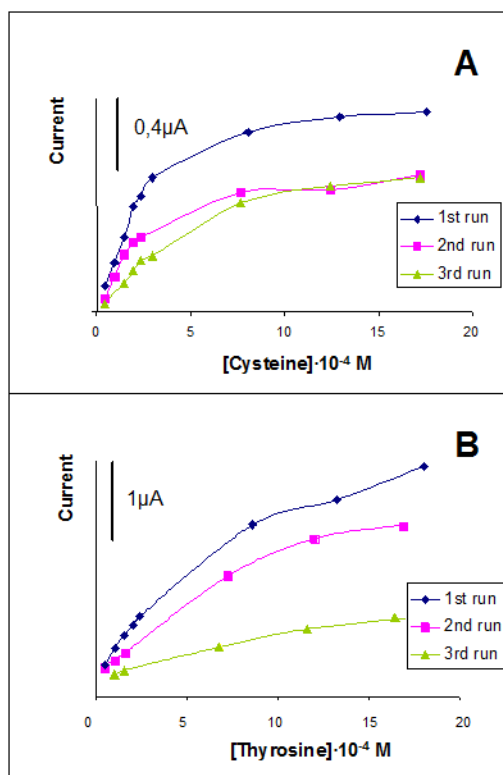
respectively. The results indicate that SPES provide good reproducibility towards oxidation of both amino acids.

Cysteine and tyrosine were determined in some pharmaceutical samples randomly taken in the market, using amperometry in stirred solution with the multiple standard addition method. Table 2 summarizes the results obtained from amperometric determination of cysteine and tyrosine along with the certified values of the analyzed pharmaceutical products. The consistent results suggest that SPES are very reliable and sensitive enough for the determination of these amino acids in real samples.

**Table 2** Results obtained for the determination of cysteine and tyrosine in pharmaceutical products using SPES. Certified values in mg or mol/ L as they appear in the corresponding product certificates are also presented (Vasjari et al, 2005)

Product	Product specifications	SPES	Certified value
Tyrosine	Eltroxin (GlaxoSmithKline, Poland)	0.093± 0.010 mg	0.1 mg
Tyrosine	Vobenol (Lek, Slovenia)	0.090±0.010 mg	0.1 mg
Cysteine	Mucotreis (Ecobi Farmaceutici, Italy)	0.297±0.040 mol/L	0.28 mol/L

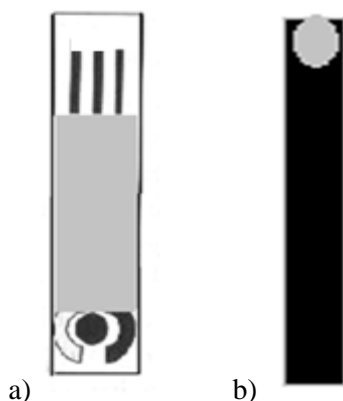




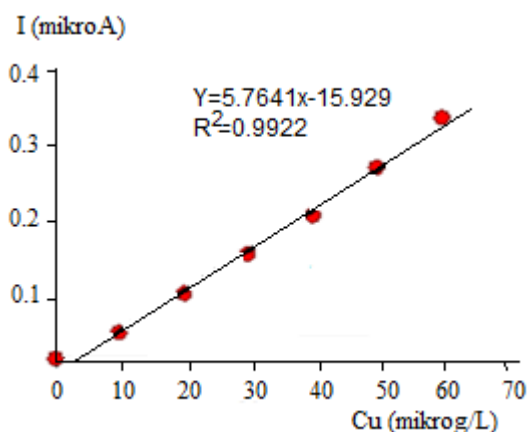
**Fig 11.** Typical amperometric calibration curves for cysteine (A) and tyrosine (B) at the optimal oxidation potentials of 0.6 and 0.8V respectively. (Vasjari et al, 2005)

## ii) Screen-printed electrodes for determination of heavy metals

Screen printed electrodes are used to determine heavy metals in sea water sample. Carbon ink working electrode, **Ag/AgCl/Cl<sup>-</sup>** as a reference electrode **and the graffite counter electrode** were printed in the same strip (Fig 12 a). Application of electrochemical methods using sreen-printed electrodes (SPE), offer several important advantages in deremination of heavy metals regarding simplicity, faster analyses and cost. Firstly the optimization of pH value and the experimental parameters of ASV were optimized (Karaj et al, 2011). It was used the optimized pH value in the cell to have highes sensitivity and to save the optimal state of electrode surface. Succesive experiment using the same SPE shown that the sensitivity and the linear working range decrease.

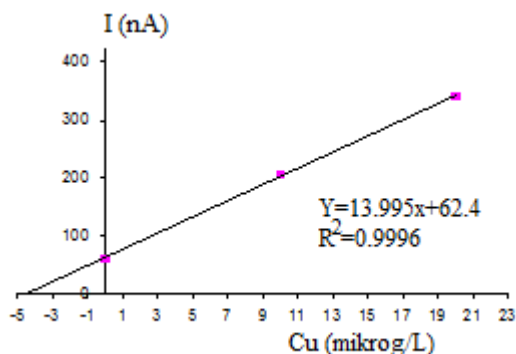


**Fig 12.** Configuration of the SPE a) three electrode system in a single strip (Carbon ink working electrode,  $\text{Ag/AgCl/Cl}^-$  as a reference electrode and Graffite counter electrode) for determination of Cu in sea water sample; b) SPE modified with rhenium (IV) oxide (working electrode) for determination of histamine in food samples.



**Fig 13.** Calibration graf for determination of Cu by SWV (period SW 250 ms, amplitude SW 30 mV,  $E_d = -0.8$  V,  $t_d = 3$  min, buffer citrat 0.1 M pH=4.5 (Karaj et al, 2011).

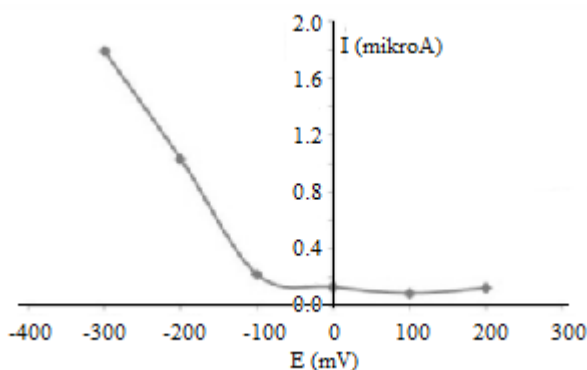
There are well known the advantages of voltametric methods for determination of HM in sea water samples related with the matric of the samples and high salinity. Determination of Cu in sea water samples of Jon Sea near Saranda, Albania is done using the method of standart additions applying the optimal experimental parameters Fig 14. Quality control of the analytical result in determination of Cu (4.45ppb), was based on based on the recovery of analyte standard additions (99.5%).



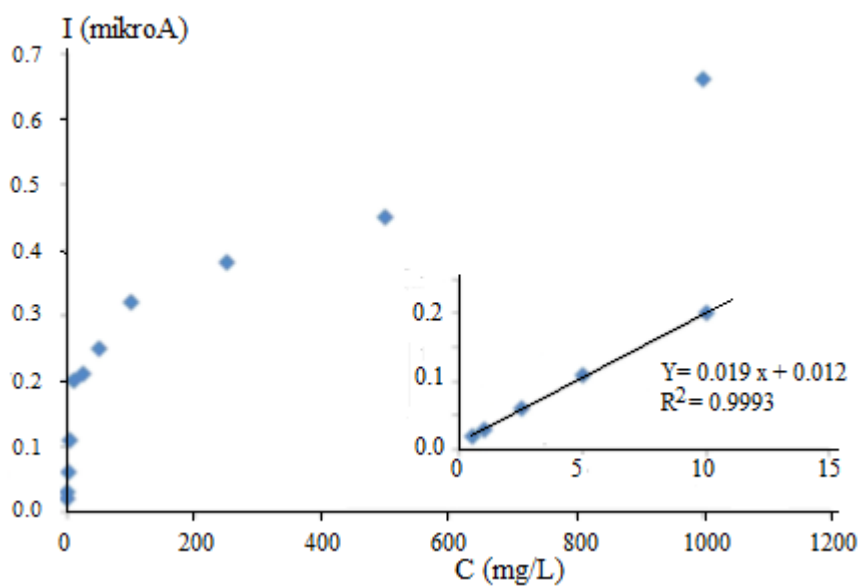
**Fig 14.** Determination of Cu in sea water sample based on standard addition method. (Karaj et al, 2011).

### iii) Screen-printed electrodes for determination of histamine

Histamine is one of the most active biogenic amine and most frequently involved in food-borne intoxications. Biogenic amines are considered as fresh food indicators. Simple electroanalytical method is developed for determination of histamine using a screen printed carbon electrode modified with rhenium oxide (IV) in batch and flow injection analysis. Modified screen printed electrodes are prepared using the homogenized mixture of 0.05g  $\text{ReO}_2$  with 1g carbon powder and 360  $\mu\text{L}$  paraffin. The printed electrodes according to procedures given in Veseli et al, (2016) were dried at room temperature overnight and then broken into individual sensor strips. The operation potential in flow injection experiments represents an essential parameter for the performance of the sensor related to the background current and possible interferences. Based on the dependence of peak height from the operation potential (Fig. 15), most suitable potential was  $-100 \text{ mV}$  which is used in the following experiments for determination of histamine. The analytical performance was evaluated based on calibration curves repeated in the same experimental conditions (Fig. 16). The detection limit was  $0.2 \text{ mg/L}$  estimated from the standard deviation of FIA-peaks at  $2.5 \text{ mg/L}$  histamine. The repeatability of measurements for  $2.5 \text{ mg/L}$  was about 4% RSD ( $n = 5$  measurements), and the reproducibility for  $2.5 \text{ mg/L}$  at different working electrodes ( $n = 5$ ). The proposed method, using screen printed carbon electrodes modified with  $\text{ReO}_2$  in FIA mode, is rapid because the sample can be applied directly after proper dilution without any further refinement steps. No additional cleaning processes of the electrode surface are necessary. The method was applied successfully to the determination of histamine in fish sauce.



**Fig 15.** Dependence of the peak height of histamine on the working potential in FIA with SPCEs modified with  $\text{ReO}_2$ ; flow rate 0.6 mL/min, carrier 0.1 M phosphate buffer, pH 7.5, histamine solution 100 mg / L injection volume 200  $\mu\text{L}$ . Veseli et al, (2016)

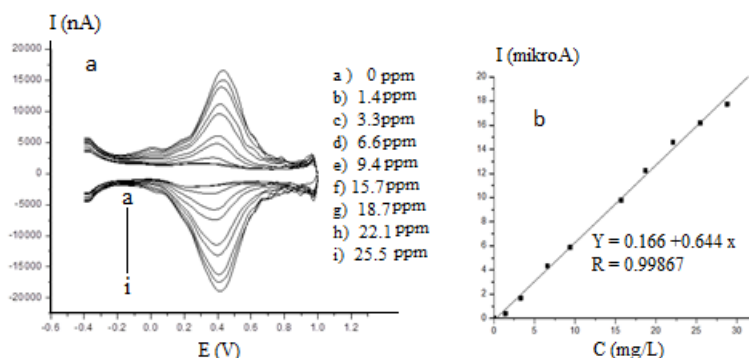


**Fig. 16.** Calibration curve for histamine at  $-100$  mV. Supporting electrolyte phosphate buffer 0.1M, pH 7.5; flow rate 0.6 mL/min; 100  $\mu\text{L}$  injection volume. (Veseli et al, 2016)

### Home- made biosensors modified with crude tissue of plants and Au-np for determination of phenolic compound

Carbon paste electrodes (CPEs) are widely applicable in electrochemical studies due to their low background current (compared to solid graphite or noble metal electrodes), low cost, feasibility to incorporate different substances during the paste preparation (in the case of modified carbon paste

electrodes), easy preparation and simple renewal of their surface and possibilities of miniaturization. Carbon paste electrodes modified with different plants tissue as source of PPO, the influence of carbon powder granulometry on the biosensors response for determination of phenolic compounds has been reported in Parroj and Vasjari (2011), Broli and Vasjari (2013). The development of a double modified sensor using the enzyme and/or Au-np is develop. To highlight the improvements in analytical performance of the CPE due to the presence of modifiers (enzyme and/or Au-np) the experiments have been conducted using unmodified CPE / CPE modified with enzyme (crude mushroom tissue) / CPE modified with Au-np / CPE modified with enzyme (crude mushroom tissue) and Au-np . The reference electrode was Ag/AgCl/Cl<sup>-</sup> and a platinum wire as auxiliary electrode. The working electrodes was prepared according the procedures published at Parroj and Vasjari (2011). The effect of incorporated modifiers to sensor response was tested by comparing the voltammograms obtained in the same experimental conditions and the calibration curves shown in Fig 17 a,b).



**Fig 17.** a) SCV using CPE modified with PPO and Au np in different concentration of catechol; b) Typical calibration graph derived from the voltamograms (Vasjari and Broli, 2014)

**Table 3** Parameters of analytical performance of the CPE, CPE modified with PPO, CPE modified with Au np, CPE modified with PPO and Au np.

	Sensitivity ( $\mu\text{A/ppm}$ )	Linear range (ppm)	(R)	Limit of detection (ppm)	$S_D$ (ppm)
CPE	0.3014	1.4-15.7	0.99558	1.24	0.6324
CPE-Enzyme	0.4218	1.4-15.7	0.99743	0.73	0.5517
CPE-Au	0.5556	1.4-25.5	0.99663	0.18	0.1977
CPE-Enzyme-Au	0.6443	1.4-25.5	0.99867	0.11	0.4134

In tab 3 are summarized the parameters of performance of the developed sensors when catechol is the testing analyt. The sensitivities toward catechol are listed as the following range CPE-Enzyme-Au > CPE-Au > CPE-Enzyme > CPE. Linear interval of the sensor response is wider when the Au-np are used as modifier of the CPE. Limit detection of catechol is improved in the case of using Au-np as modifiers. CPE modified with Au-np has better analytical performance towards phenolic compounds

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## SENSORS AND BIOSENSORS DEVELOPMENT FOR APPLICATION IN ENVIRONMENTAL AND FOOD SAMPLES

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### ABSTRACT

Carbon paste electrode modified with different kind of plant tissue and/or gold nanoparticles is studied. The crude tissues of different plant such as banana (CPE-B), apple (CPE-A), mushroom (CPE-M), potato (CPE-Po) and pear (CPE-Pe) as a source of PPO enzyme and/or Au nanoparticles are used for modification of carbon paste electrode. The ratio between components is optimized based on electrochemical response of biosensors using voltammetric technique. The analytical performances of biosensors are evaluated versus catechol analyte. The CPE-PPO/AuNPs electrode resulted with the highest analytical performance: sensitivity  $8 \times 10^{-7} \mu\text{A/mol/L}$ , correlation coefficient 0.9999 and detection limit  $1.8 \times 10^{-7} \text{ mol/L}$ . Homemade carbon paste electrode modified with gold nanoparticles (AuNPs-CPE) is used for the determination of Hg(II) using anodic stripping voltammetry. Under optimal experimental conditions the voltammetric signals were linearly depended on the Hg (II) concentration in the range of  $7.5 \times 10^{-7} \text{ mol/L}$  to  $2.99 \times 10^{-7} \text{ mol/L}$ , with sensitivity  $33.1 \times 10^{-5} \mu\text{A/mol/L}$  and detection limit  $1.5 \times 10^{-7} \text{ mol/L}$ . The modified electrodes CPE-PPO/AuNPs, CPE-B and CPE-Au/NPs were tested in environmental and food samples. The obtained results showed that proposed sensor and biosensors can be used for a rapid determination of phenolic compounds and Hg (II) in real sample (green tea, coffee and underground water).

**Keywords:** Biosensors, voltammetric technique, PPO enzyme, gold nanoparticles, crude tissue.

### 1. INTRODUCTION

Phenols and substituted phenols are important contaminants in medical, food and environmental matrices. In view of their high toxicity, reliable analytical procedures are required for sensitivity determination at low level in various matrices (Negash *et al*, 2014). Electrochemical methods have been widely used for measuring these compounds due to their advantages such as good selectivity in the presence of phenol oxidases, relatively low cost of

realization and storage, the potential for miniaturization and automation. Among heavy metals, mercury metal, which is found in biological materials, natural water, soil, air, chemicals and waste products, is highly toxic, resulting in interference and disturbance of natural systems as well as the production of damaging effects to the environment so the determination of mercury in environmental samples is therefore very important (Chey *et al*, 2012). Carbon paste electrodes (CPEs) are widely applicable in electrochemical studies due to their low background current (compared to solid graphite or noble metal electrodes), low cost, feasibility to incorporate different substances during the paste preparation (in the case of modified carbon paste electrodes), easy preparation and simple renewal of their surface and possibilities of miniaturization. Recently the preference for using vegetable or plant tissue instead of purified enzymes, directly without any pretreatment is reported (Cummings *et al*, 1998). This group of biocatalyst materials maintains the enzyme of interest in its natural environment. Many fruits and vegetables are rich in enzymes and can be used in biosensors construction. Carbon paste electrode could be modified with **crude tissues** of different plants (source of PPO enzyme) such as: banana, apple, mushroom, pear, potato etc, in order to improve their electrochemical performance. PPO enzyme catalyses the oxidation of o-diphenols to o-quinones by consumption of molecular oxygen. These quinones are electrochemically reduced at a low potential and the measured current is proportional to the phenolic compound concentration. The use of gold nanoparticles (Au-np) immobilized together with an enzyme in an electrode membrane have been shown to improve the response of the enzyme electrode where gold nanoparticles of small size allow more freedom in the orientation for the anchored protein molecules and hence maximize the utilization of their bioactive sites (Silvia *et al*, 2005). In this study we report the development of a double modified sensor using the enzyme and the Au-np. Carbon paste electrode modified with PPO enzyme, crude tissue of different plant and gold nanoparticles are used as a working electrode in voltammetric experiments on the response of electrochemical sensor and biosensors for detection of phenolic compound and mercury.

## 2. MATERIALS AND METHODS

### *Reagents and chemicals*

All solutions were prepared using deionized water and all chemicals were of analytical grade and used without further purification. Carbon graphite powder synthetic, (99.9% Alfa Aesar with particles size 0.09-0.071 mm) and paraffin oil (Olio di Vaseline, Zeta farmaceutici) were used for the preparation of the carbon paste. The crude tissue of mushroom, apple, potato, pear and banana peel was used as source of PPO and purchased from a local

market as fresh culture fruit. Polyphenol oxidase enzyme (PPO Units 25000). Standard solution Hg (II) (Aldrich) (1000 mg/L) and the low concentration standard solutions of mercury were freshly prepared before the measurements. Phenol, 4-Chlorophenol, m-Cresol, p-Cresol and Hydroquinone were purchased from Merck 99%. Stock solution of catechol (99 % Alfa Aesar) (5M) was prepared in 0.1 M phosphate buffer of pH 7.0. Phosphate buffer solution 0.1 M was prepared by mixing suitable amounts of  $K_2HPO_4 \cdot 3H_2O$  and  $KH_2PO_4$ ,  $K_2HPO_4$ , KCl (99 % Merck).  $H_3PO_4$  (1:9) was prepared: 10 ml  $H_3PO_4$  with 90 ml doubly distilled water, for water sample pretreatment. The supporting electrolyte pH=2 was prepared by mixing of 10 ml  $K_2HPO_4$  0.4 M solution with 50 ml citric acid 0.25 M (99 % Merck) and 10 ml KCl 0.5 M (99.5%, Normapur). The colloidal solution of gold nanoparticles was prepared by reduction of  $HAuCl_4$  (0.96%) solution with  $NaBH_4$  1 M (96% Merck).

### ***Electrochemical measurements***

Electrochemical experiments were performed with an electrochemical analyzer (**MEC-12B**) using a three electrode system. Carbon paste electrode (CPE), CPE modified with crude tissue of (banana, apple, mushroom, pear, potato), CPE modified with Au-NPs, CPE modified with PPO enzyme+Au-NPs as the working electrode, a platinum wire was used as a counter electrode with a saturated calomel reference electrode (**Ag/AgCl**), completing the cell assembly. All experiments were carried at room temperature. Cyclic voltammograms with SWV were obtained at potentials scanning from  $E_i = -0.4$  V to  $E_f = 1.0$  V, in 0.1 M buffer solution (pH= 7), scan rate 50 mV/s. The anodic stripping voltammetry was recorded from -0.2 V to 0.6 V in buffer solution (pH 2.7). Standard solutions of Hg (II) were accumulated at 0.16 V for 60 s and 120 s while stirring the solution. In each case the background voltammogram was firstly recorded and then the addition of analyte was introduced into the cell, kept under stirring with a magnetic stirrer.

### ***Preparation of CPE and modified CPEs***

*The modified carbon paste sensors (CPE-B, CPE-A, CPE-M, CPE-Pe, CPE-Po)* were prepared by mixing firstly the graphite powder (0.09-0.071mmgranulometry) and paraffin (280 $\mu$ l) and then the modifier (crude tissue of banana, apple, mushroom, pear and potato) was added and mix for at least 20 min to obtain a homogenous paste. Unmodified carbon paste electrode was prepared in the similar way without using a modifier (Broli and Vasjari, 2011, 2012).

*The CPE modified with gold nanoparticles (CPE- Au-NPs).* The gold nanoparticles were prepared according to (Broli and Vasjari. 2015). The carbon paste electrode containing gold nanoparticles (CPE-Au-NPs) was prepared by mixing in an agate mortar graphite powder (1.5 g) with 2 ml

colloidal gold solution, followed by the incorporation of the mineral oil (100  $\mu$ L) and mixing for additional 20 min.

*The CPE with gold nanoparticles and PPO enzyme (CPE-PPO/AuNPs)* was prepared in the following way: Three milligrams of PPO was dissolved in 300  $\mu$ L phosphate buffer 0.1M (pH 7.0), and then (200  $\mu$ L) of this enzyme solution was added to the carbon paste electrode containing gold nanoparticles (CPE-Au-NPs) (Broli and Vasjari, 2015) and mixed well to obtain a homogenous paste. The resulting paste was packed into a plastic syringe containing a copper wire as the external electric contact. A new surface was obtained by smoothing the electrode in a clean glass surface before the measurements.

### ***Samples Preparation and Measurement***

*Underground water* samples were cleaned by undergoing a distillation process to minimize interference during electrochemical determination of phenol. It was built distillation apparatus according to the instructions in the manuals of use of the equipment (*Salgado and Simoes 2013*). In 500 ml water sample taken in cylinder and passed on to the balloon distillation was added 10 %  $\text{H}_3\text{PO}_4$  drops to adjust pH = 4 (for the elimination of sulfur compounds that are released in the form of  $\text{H}_2\text{S}$  and  $\text{SO}_2$ ). After collecting 500 ml of distillate, the samples are ready to be analyzed using CPE-B biosensor optimized in laboratory conditions. For analysis 5 ml sample was added into electrochemical cell containing 15 ml of 0.1M phosphate buffer (pH = 7). Studied phenols are very volatile and the samples were analyzed within a short time and for how long are used they are stored in the refrigerator.

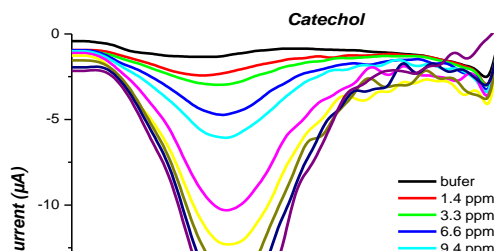
*The green tea and coffee* sample was purchased in casual market. The sample was prepared by extracting 1.0 g of the tea with 50 ml of the 0.1 M phosphate buffer solution (pH 7.0) at 35  $^{\circ}\text{C}$ , incubating for 20 min, shaking for 2 min and filtering. An aliquot of 0.1 ml of the green tea extract was transferred to an electrochemical cell containing 15 ml 0.1M phosphate buffer solution and analyzed after successive additions of the standard catechol solution. After each addition, voltametric measurements were recorded from -0.4 to 1.0V. All measurements were performed in triplicate.

## **3. RESULTS AND DISCUSSION**

### ***Electrochemical Response of the modified Electrodes***

It was prepared a carbon paste electrode modified with crude tissue of banana **CPE-B**. Under the optimized conditions (Broli and Vasjari, 2011), the variation of peak current with concentration of catechol at the CPE-B was studied by Square Wave Voltammetry and the result is shown in fig. 1. From the figure we can see that the reduction peak current of catechol at the CPE-B

was proportional to its concentration with correlation coefficient of 0.9981, sensitivity  $2.32 \mu\text{A}/\text{mg}/\text{L}$ , limit of detection ( $S/N = 3$ ) of  $0.1 \text{ mg}/\text{L}$ .



**Fig. 1:** Square Wave Voltammograms in 0.1M phosphate buffer solution pH 7; amplitude 30 mV and step potential 10 mV. (Broli and Vasjari 2015).

The reproducibility expressed in relative standard deviation ( $n=4$ ) was found to be (RSD 3.6%), and repeatability (RSD 3.2%).

The electrochemical behavior of carbon paste biosensor modified with apple tissue **CPE-A** was tested towards: catechol, phenol, chloro-4-phenol, hydroquinone, p-cresol, m-cresol, amino-3-phenol, nitro-3-phenol, nitro-4-phenol. In table 1 it was shown the analytical parameters of biosensor such as sensitivity, detection limits, relative response, etc.

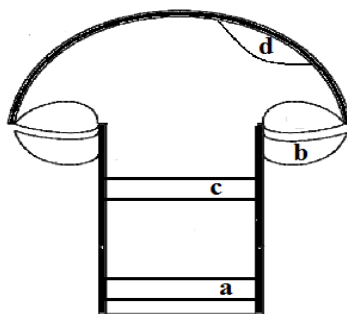
**Table. 1** Analytical performance of CPE-A biosensor in different phenolic compounds

Analyte	Sensitivity b ( $\mu\text{A}/\text{mg}/\text{L}$ )	Limit of detection ( $\text{mg}/\text{L}$ )	Linear Range ( $\text{mg}/\text{L}$ )	Relative Response (%)
<b>Chloro -4-phenol</b>	1.35	0.48	1.4-9.4	100
Phenol	1.18	1.01	1.4-6.6	87
Catechol	1.17	1.80	3.3-6.6	86
Hidroquinone	0.59	4.00	3.3-9.4	61
p-Cresol	0.48	2.60	3.3-6.6	31

The different responses between those phenolic compounds are due to the different substituent groups and their position. Maximum sensitivity was

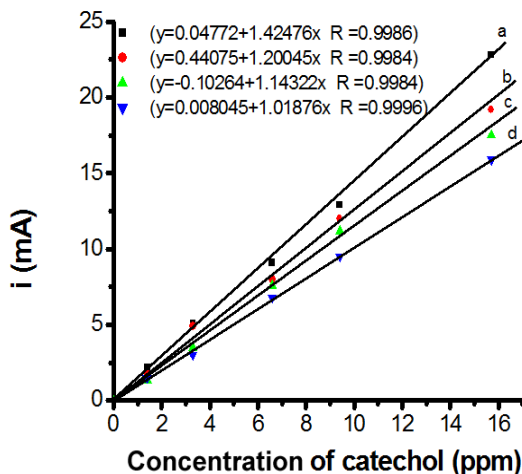
found to be  $1.35 \mu\text{A}/\text{mg}/\text{L}$  for **chloro-4-phenol**. The relative response of the CPE-A biosensor in the same experimental conditions is calculated considering the response in chloro-4-phenol 100%. The different sensitivities varied between  $0.48\text{--}1.35 \mu\text{A}/\text{mg}/\text{L}$  for the tested phenolic can be related to the formation of o-quinones during the enzymatic reaction (Ozoner *et al.*, 2011).

In several papers is reported, the distribution of PPO enzymes activity is not uniform in different morphological part of mushroom. There are prepared (Broli and Vasjari, 2013) and experimented carbon paste electrodes modified with crude tissue taken from different sections in accordance to the mushroom (CPE-M) schematic shown in Fig. 2



**Fig. 2:** Different part of mushroom used as sensor modifier: a) bottom of stem, b) gill, c) upper of stem, d) cap. (Broli and Vasjari 2013).

The electrochemical behavior of experimented biosensors was studied based on the voltammogrammes (SWV) and corresponded calibration graphs obtained in each case. The measurement was performed using catechol as a substrate in phosphate buffer 0.1 M, pH 7. In figure 3, it is obviously shown that the source of modifier resulted in changes in the calibration curves sensitivity.



**Fig. 3.** Calibration curves of CPE-M using a.b.c.d section as modifier,  $E = 0.4$  V, scan rate 50 mV/s. (Broli and Vasjari, 2013).

The stalk tissue (**section a**) appears to contain more active enzyme than other studied morphological parts. The results obtained here showed that the enzyme activity is determined by its living environment and is very important the incorporation step into the carbon paste do not affect in its activity. The response of the CPE-M to phenol, cloro-4-phenol, hydroquinone, catechol, p-cresol, m-cresol, 4-aminophenol, 3-nitrophenol, 4- nitrophenol was investigated. No response was obtained towards 4-aminophenol. Table 2 summarizes the characteristics of the calibration plots for the studied phenol derivatives, as well as the sensitivity, detection limit and standard deviation.

**Table 2.** Analytical parameters of biosensor in various phenolic compounds.

Analyte	Sensitivity ( $\mu\text{A}/\text{mg/L}$ )	$R^2$	Detection limit ( $\text{mg/L}$ )	Standard deviation	Relative response (%)
Hydroquinone	2.04	0.9997	0.75	0.31	100
Catechol	1.27	0.9986	1.77	0.44	62
Phenol	1.12	0.9541	9.56	2.29	55
m-Cresol	0.98	0.9659	4.52	1.16	48
Chloro-4-phenol	0.95	0.9979	1.37	0.4	47
p-Cresol	0.79	0.9650	5.58	1.39	39
Nitro-4-phenol	0.77	0.9921	2.63	0.64	37
Nitro-3-phenol	0.70	0.9405	6.53	1.64	34
Amino-4-phenol	no response	-	-	-	-

The detection limit ranged between 0.75 and 9.56 mg/L for the tested phenol derivatives. The maximum sensitivity was found to be 2.04  $\mu\text{A}/\text{mg}/\text{L}$  for hydroquinone. The CPE-M biosensor showed relatively high sensitivity for catechol ( $S = 1.27 \mu\text{A}/\text{mg}/\text{L}$ ). This can be related with the presence of  $-\text{OH}$  group of catechol in ortho position which enhances oxidation of the o-diphenol to quinone by PPO enzyme. Phenolic compounds regarding sensitivity of CPE-M are listed as the following range: hydroquinone > phenol > m-cresol > catechol > chloro-4-phenol > p-cresol > nitro-4-phenol > nitro-3-phenol.

### ***Summary of analytical performance toward catechol***

It is interesting to compare the performance of different sensor and enzymatic biosensor, so table 3 summarizes the characteristics of the calibration plots using catechol as analyte.

**Table 3.** Analytical parameters for modified carbon paste electrodes

Parameters	CPE-M	CPE-B	CPE-A	CPE-Pe	CPE-Po	CPE
Measurements (n)	4	4	3	3	3	3
Standard Deviation (mg/L)	0.26	0.08	0.70	0.97	0.40	1.15
Relative standard deviation (%)	4.9	3.2	9.3	22	11,1	47
Linear range (mg/L)	1.4-15.7	1.4-15.7	3.3-6.6	3.3-6.6	3.3-6.6	3.3-6.6
Sensitivity ( $\mu\text{A}/\text{mg}/\text{L}$ )	1.54	2.32	1.17	0.95	0.91	0.60
Limit of detection (mg/L)	0.5	0.1	1.8	3.1	1.3	5.7

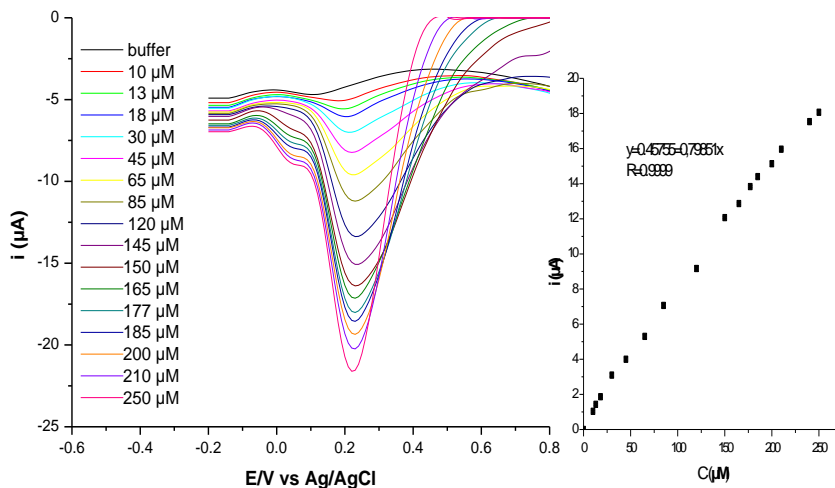
As it can be seen from table the **CPE-B** resulted with the best analytical parameters than other biosensor, regarding sensitivity, limit of detection, linear range, standard deviation (Broli and Vasjari, 2013).

### ***The Analytical performance of CPE-PPO/AuNPs Biosensor***

Using CPE-PPO/AuNPs, the optimal square wave parameters and the calibration curve for the determination of catechol concentration was established (Broli and Vasjari, 2014). The peak height for catechol was found



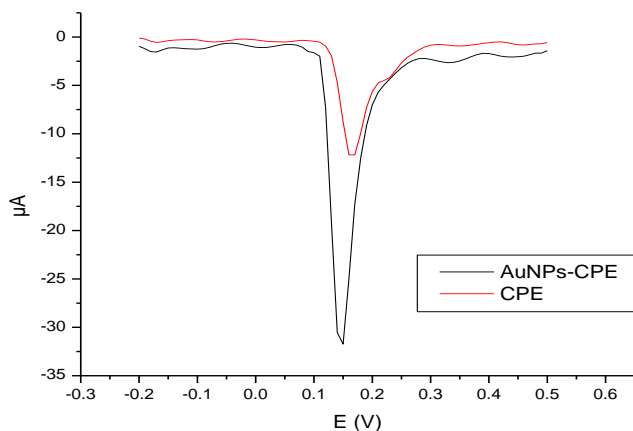
to increase with increasing concentration from 10 to 250  $\mu\text{M}$  (Figure 4) ( $n = 16$ ). As it is indicated the data sets showed a good linear fit because the value of  $R^2$  is approaching positive one (0.9999). The detection limit (three times the standard deviation of 10  $\mu\text{M}$  catechol/slope) was calculated using this calibration curve, and found to be 0.18  $\mu\text{M}$  respectively. Reproducibility of the signal obtained in 10  $\mu\text{M}$  catechol resulted with relative standard deviation 5.52 % ( $n=3$ ).



**Figure 4.** Square wave voltammograms of CPE-PPO/AuNPs biosensor in different concentration of catechol, amplitude 30 mV and step potential 10 mV. (Broli and Vasjari, 2014)

### ***Carbon Paste Electrode modified with Gold nanoparticles***

Anodic stripping voltammetry was used to study the electrochemical behavior of the bare carbon paste electrode **CPE** and modified electrode **AuNPs-CPE** in a stirred buffer solution (pH 2.7).

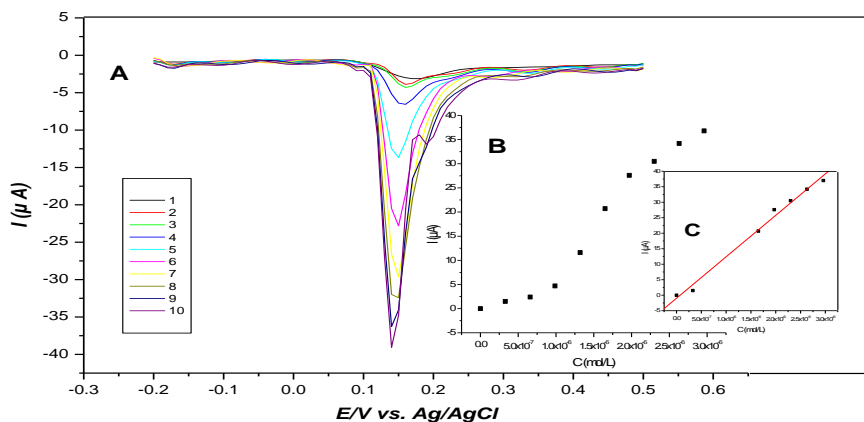


**Fig. 5.** Anodic stripping voltammograms in  $2.9 \times 10^{-6}$  mol/l  $\text{Hg}^{2+}$ . The red line displays the CPE and the black line the AuNPs-CPE in buffer solution pH=2.7, for an accumulation time of 120 s at -0.2 V and a scan rate 50 mV/s. (Broli and Vasjari, 2015).

The anodic stripping voltammograms of the AuNPs-CPE (black line) and the carbon paste electrode CPE (red line) in the presence of  $2.9 \times 10^{-6}$  mol/l  $\text{Hg}^{2+}$  is shown in Fig. 5. In the modified electrode an enhancement of the anodic peak current at a potential of 0.16 V was observed in comparison with the CPE. The signal obtained with CPE was lower than signal obtained with modified electrode this can be attributed the catalytic effect of colloidal gold nanoparticles. The amount of modifier (gold nanoparticles) in the carbon paste was optimized by comparing the stripping peaks of current obtained in the same concentration of mercury ( $2.9 \times 10^{-6}$  mol/l), using carbon paste with different mass ratio between graphite powder, gold nanoparticles and paraffin (Broli and Vasjari, 2015). The presence of the gold nanoparticles in CPE greatly improves the peak current of Hg red- oxidation.

### Analytical Performance of AuNPs-CPE Sensor

Under the optimized conditions ( $t_d=120$  sec,  $E_d=-1\text{V}$ ), the relationship between the anodic peak current and the Hg (II) concentration was studied. As it is show in figure 6 the peak current increases proportionally with the Hg (II) concentration to yield a linear calibration plot (Fig 6.C) with sensitivity  $33.1 \times 10^{-5}$   $\mu\text{A/mol/L}$ , correlation coefficient  $R^2= 0.9952$  and the low detection limit (LOD) of Hg (II)  $1.5 \times 10^{-7}$  mol/L.



**Figure 6.** (Inset A) Anodic stripping voltammograms using **AuNPs-CPE** in buffer solution (pH 2.7),  $t_d=120$  s,  $E_d=-1V$ , concentration of  $Hg(II)$ : (1) blank; (2)  $3.29 \times 10^{-7}$ ; (3)  $6.58 \times 10^{-7}$ ; (4)  $9.87 \times 10^{-7}$ ; (5)  $1.32 \times 10^{-6}$ ; (6)  $1.65 \times 10^{-6}$ ; (7)  $1.97 \times 10^{-6}$ ; (8)  $2.30 \times 10^{-6}$ ; (9)  $2.63 \times 10^{-6}$  and (10)  $2.96 \times 10^{-6}$  mol/l. (Inset B) Plot of catalytic current versus concentration of  $Hg(II)$ . (Inset C) Linear calibration curve (Broli and Vasjari, 2015) .

The relative standard deviation (RSD) of less than 5.0% was obtained for five repeated measurements of  $3.7 \times 10^{-7}$  mol/l  $Hg(II)$  solutions.

## ANALYTICAL APPLICATIONS

### 1. Analysis of catechol in tea samples using CPE-PPO/AuNPs

By detecting the ability **CPE-PPO/AuNPs** biosensor to detect catechol in green tea, we verified its suitability for practical application. The content of catechol was determined with SWV using standard addition method, and the result is given in the Table 4.

**Table 4.** Determination of catechol in green tea using CPE-PPO biosensor. (n=3)

Sample	Catechol ( $10^{-3}$ mol/L)
(Green tea)	$5.0 \pm 0.2$

In order to evaluate the effect of the presence of potential interferences on the proposed biosensor, the standard additions method was applied. Analytical recovery measurements were obtained by adding different amounts of catechol ( $16$  to  $33 \times 10^{-6}$  mol/L) to sample of green tea. The percentage recovery values were calculated by comparing the concentration obtained

from the samples with and without the addition of known concentrations of the catechol standard solution.

**Table 5.** Results for recoveries of catechol standard solution from green tea using CPE-PPO biosensor.

Catechol ( $10^{-6}$ mol/L) (n=3)			
Sample	Added	Found	Recovery (%)
(Green tea)	16	15.4±0.1	96
	33	33.4±0.1	101

It can be clearly observed that the recovery results obtained suggest an absence of matrix effects in these determinations.

## 2. Determination of phenolic compounds and catechol using CPE-B

Based on the electrocatalytic effect of banana tissue (natural PPO enzyme), the proposed electrode was applied for determination of phenolic compound and catechol in real sample. The concentration of catechol in drinking coffee with SWV using standard addition method (n=3), was  $2.3 \times 10^{-3} \pm 0.4$  mol/L. The calculated recoveries are listed in table 6.

**Table 6.** Determination results of catechol in coffee (n=3)

Sample	Added ( $10^{-5}$ mol/L)	Found ( $10^{-5}$ mol/L)	Recovery (%)	RSD (%)
Coffee	1.63	1.83	112	3.4
	3.27	2.36	72	4.1

As shown in Table 6 the recoveries were between 72% and 112 %, which confirm the designed electrode is reliable for quantitative determination of catechol in drinking coffee. Also the CPE-B biosensor was tested in determination of phenolic compound (hydroquinone, chloro-4-phenol, p-cresol, m-cresol, phenol) in underground water samples. The samples of underground water in phosphate buffer solution were determined using standard addition method and SWV technique in optimal conditions recommended in (Broli and Vasjari, 2015) (Table 7).

**Table 7.** Experimental results obtained by electrochemical detection of phenols in underground water sample (n=3)

Underground water samples Amount (mol/L)			
Chloro-4-phenol	p-Cresol	m-Cresol	Phenol
$1.9 \times 10^{-5} \pm 0.02$ mol/L	$2.2 \times 10^{-5} \pm 0.1$ mol/L	$1.8 \times 10^{-5} \pm 0.02$ mol/L	$3.6 \times 10^{-5} \pm 0.1$ mol/L

The results demonstrate and confirm the capability of the modified electrode (CPE-B) for the voltammetric determination of phenol, chloro-4-phenol, p-cresol, m-cresol with good selectivity and good reproducibility. In order to evaluate accuracy of the method and the presence of systematic errors the recovery method was used. Analytical recovery measurements were calculated based on the results obtained by adding different amounts of phenols (chloro-4-phenol, p-cresol, m-cresol and hydroquinone) into the sample. In table 8 are shown the recoveries results.

**Table 8.** Determination of phenols in underground water samples using the CPE-B Biosensor.

Analyte	Amount added (mol/L)	Found <sup>a</sup> (mol/L)	RSD <sup>b</sup> (%)	Recovery (%)
Hydroquinone	$4.99 \times 10^{-6}$	$4.90 \times 10^{-6}$	2.77	98.2
	$9.99 \times 10^{-6}$	$9.18 \times 10^{-6}$	2.02	91.8
Chloro-4-phenol	$4.98 \times 10^{-6}$	$4.85 \times 10^{-6}$	8.73	97.4
	$9.96 \times 10^{-6}$	$10.03 \times 10^{-6}$	8.40	100.7
m-Cresol	$4.99 \times 10^{-6}$	$4.80 \times 10^{-6}$	1.96	96.2
	$9.98 \times 10^{-6}$	$9.70 \times 10^{-6}$	4.70	97.2
p-Cresol	$4.99 \times 10^{-6}$	$4.90 \times 10^{-6}$	4.00	98.2
	$9.98 \times 10^{-6}$	$10.07 \times 10^{-6}$	8.25	100.9

<sup>a</sup>Average value of three measurements.

<sup>b</sup>Relative standard deviation for the proposed method (n = 3).

These results clearly show that our electrochemical biosensor is a reliable and effective for determinations of phenols in real samples.

### ***3. Determination of mercury by anodic stripping voltammetry with AuNPs-CPE***

In order to test practical application of *AuNPs-CPE sensor*, the Hg (II) content was determined in “sea” water samples. The concentration of Hg (II) in the water samples was determined by the standard addition method to compensate the matrix effect from the sample. Regarding about the concentration of Hg (II) in sea water sample was found  $1.2 \times 10^{-7} \pm 0.001$  mol/L (n=3). The quality control of this analytical result was tested evaluating the recoveries of the Hg (II) additions in samples.

**Table 9.** Quality control of analytical results based on recovery of standard addition (n=3).

<b>Hg(II) concentration (mol/L)</b>				
Sample	Added	Found	Recovery (%)	RSD (%)
Sea Water	$4.24 \times 10^{-6}$	$4.33 \times 10^{-6}$	102.3	4.7
	$6.23 \times 10^{-6}$	$5.88 \times 10^{-6}$	99.8	3.6

The recovery (table 9) between 99.8 % and 102.3% was satisfactory thus indicate the suitability of the proposed method for applications in sea water samples (Broli and Vasjari, 2015). In addition, interference from other species in water samples could be almost negligible.

## **4. CONCLUSIONS**

Enzymatic carbon paste biosensors modified with crude tissue of plant, PPO enzyme and or/ gold nanoparticles provide analytical determinations with good selectivity and sensitivity. Analytical performances of biosensors toward catechol are listed as the following range: **CPE-B > CPE-M > CPE-A > CPE-Pe > CPE-Po > CPE**. The incorporation of polyphenol oxidase enzyme into the composite containing carbon paste and gold nanoparticles (**CPE-PPO/AuNPs**) has allowed us to obtain in a very easy and fast way a highly sensitive and selective sensor for catechols by combining the advantages of composite materials and the electrocatalytic properties of gold nanoparticles. The modified electrodes **CPE-PPO/AuNPs, CPE-B and CPE-**

**Au/NPs** can be used for a rapid determination of different analytes in real sample with good reproducibility and stability.

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## NANOSTRUCTURING GRAPHITES AND DIAMONDS FOR BIO-RELATED APPLICATIONS

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### ABSTRACT

In this review, we present here some innovative  $sp^2$  and  $sp^3$  carbon-based nanomaterials for the building of multivalent architectures and illustrate the synthetic strategies explored for the engineering of differently shaped substrates to be used in the bio-medical field. We focus, in particular, on systems formed by organized arrays of tubular graphitic structures (carbon nanotubes, helical nanotubes, dendrimers), by plasma-etched diamond layers and grains, and/or by self-assembled diamond nanograins. The tailoring of functional interfaces based on nanographites and nanodiamonds is proposed as an effective approach for the assembling of scaffolds for tissue growth and of drug-delivery platforms.

**Keywords:** carbon nanotubes, nanodiamonds, plasma deposition

### 1. INTRODUCTION

Advances in material science are nowadays providing novel approaches for the production of innovative nanostructures and are opening new opportunities to design systems for applications in a variety of technological fields. With the rapid expansion of nanotechnology, new scenarios are being opened, with promises of important developments also in the traditional bio-related disciplines. In the last few years many decisive steps have been taken towards the design of nanomaterials and systems for bio-labelling, imaging, drug delivery, tissue growth, and fabrication of miniaturized devices for health care.

For tissue growth, an emerging research field is related to the engineering of nanostructures to produce 3D complex networks that mimic the complex highly structured local microenvironment offered by natural extracellular matrices (Kiang *et al.*, 2013). 3D frameworks with multivalent architectures, the so-called grafts, scaffolds and platforms, are needed not only to assist cell

proliferation and differentiation and to organize the cells into an ordered assembly, but also to supply a rigid support for tissue growth from cells. For these purposes, it is required a material able at the same time to provide mechanical support for embedded cells and to promote a series of cellular functions, from adhesion to migration (Bacakova *et al.*, 2011).

In the last two decades, the biomedical community has realized the potentiality of the carbon nanostructures, namely carbon nanotubes, fullerenes, graphenes, and nanodiamonds, for applications in diverse areas of biology and medicine. In this regard, both  $sp^2$  and  $sp^3$  nanocarbons, characterized by good biocompatibility, high stability and long-time reliability, are now being proposed to solve the task of producing scaffolds/platforms suitable for tissue engineering. The unique properties of various classes of carbon at the nanoscale, coupled with the wide possibility of surface termination and functionalization offered by the shaping in a variety of nanostructures, make nanocarbons very attractive for bioengineering and medical researches (Bacakova *et al.*, 2016).

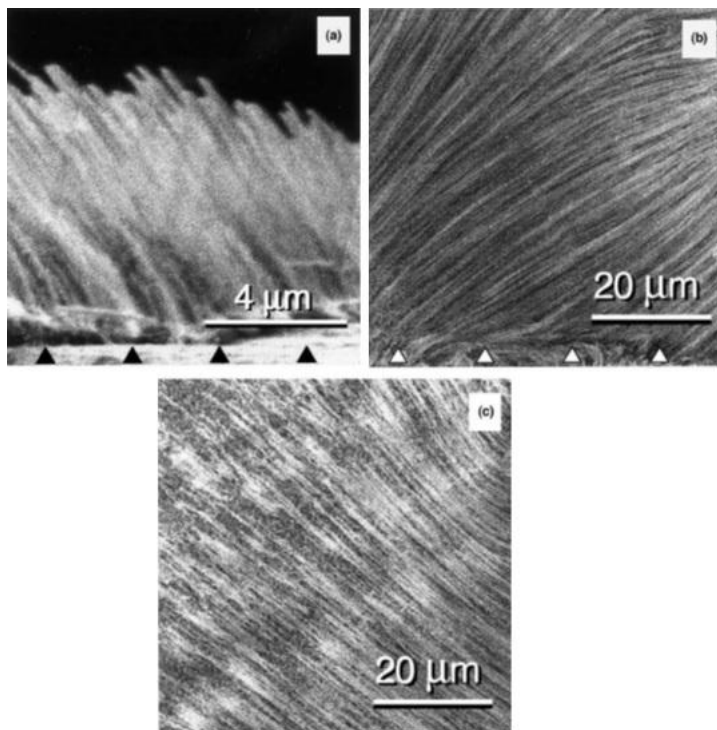
The forms of  $sp^2$  nanocarbons that can be exploited in a wide range of biomedical utilities mainly consist in: nanotubes, helical tubes, dendrimers, nanowalls and graphenic platelets. For the  $sp^3$ -hybridized carbons a variety of low-dimensional diamond materials are proposed.

Starting from the results obtained so far in our labs, this paper aims to present some interesting example of carbon nanostructures that are being prepared for bio-related applications, and to illustrate promising routes to optimize the fabrication of scaffolds and platforms.

### **$Sp^2$ NanoCarbons**

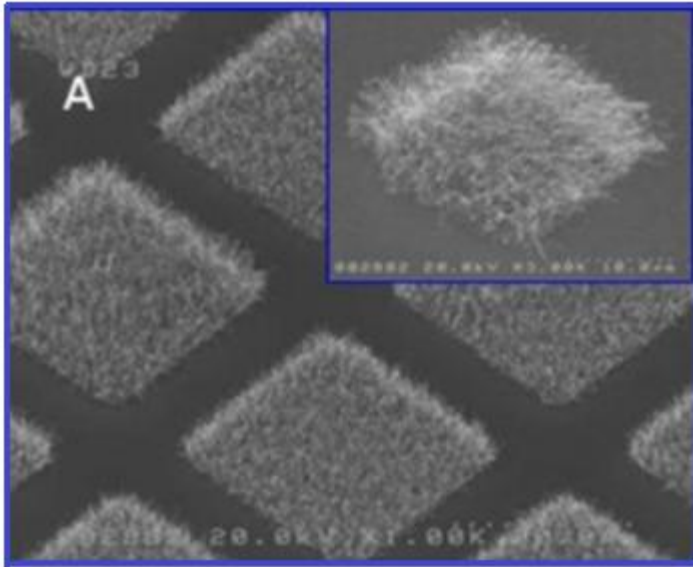
Among the wide family of nanographites, carbon nanotubes (CNT) are considered the best for fabrication of systems forming local organized microenvironment. As nanotubes are considered metastable species, all the proposed synthesis techniques consist in non-equilibrium metal catalyzed processes which involve a source of elemental carbon and a transfer of energy.

In this context, the adoption of CVD-based technologies represents the best way to prepare nanotube ensembles with specific organization. The use of purpose-designed CVD reactors makes it possible to produce ordered arrays of carbon nanotubes by reactions of gaseous hydrocarbons or carbon powders carried by inert gases, with the “in situ” generated atomic H (Terranova *et al.*, 2016). The modulation of the process parameters allows to obtain either aligned bundles of horizontally placed nanotubes laying along the substrate or dense arrays of nanotubes oriented at variable angles with respect to the substrate surface. Some examples of such organized systems can be viewed in Fig. 1.



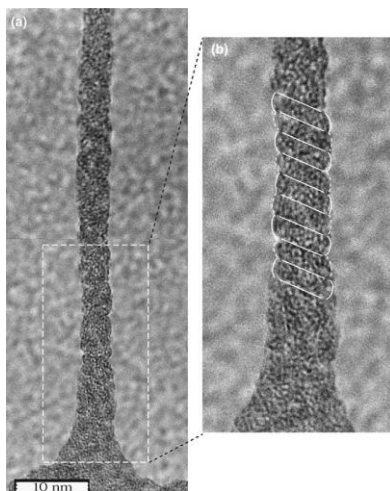
**Fig. 1.** FE-SEM images of nanotube bundles differently oriented respect to the substrate (approximately 60°, 30° and 0° in (a), (b) and (c), respectively). Adapted from Ref. (Orlanducci *et al.* 2003).

Moreover, controlled growth of packed nanotube arrays can be obtained at given locations of a substrate by means of a controlled distribution of catalysts (such as Ni, Co, Fe) using lithographic processes. Fig. 2 illustrates a FE-SEM micrograph of CNT obtained from this approach.



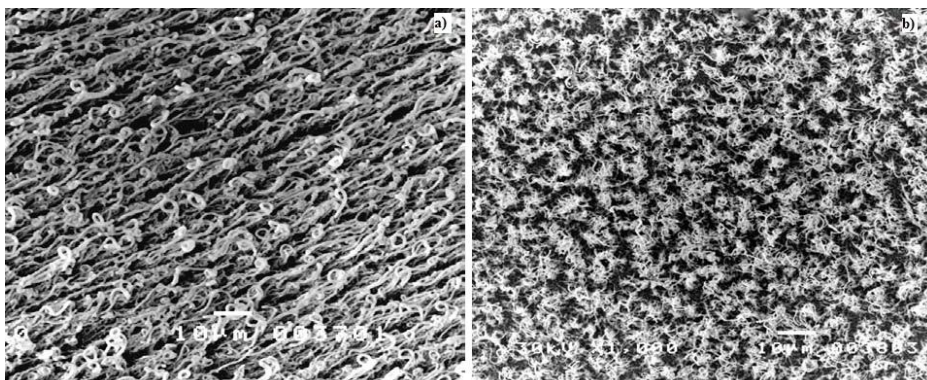
**Fig. 2.** FE-SEM images of CNT deposits on:  $10 \times 10$  squared patterned substrates. Adapted from Ref. (Guglielmotti *et al.* 2011).

The spatial control of CNT produced by CVD techniques offers many possibilities for the development of bio-active surfaces and platforms for bio-implants. Properties similar to those of CNT are also exhibited by other types of tubular nanostructures, such as dendrimers and helical nanostructures. The first ones can be produced by CVD processes using amorphous carbon powders containing traces of S in the range of ppm as precursor. Conversely, the generation of helical nanostructures depends on the spatial distribution of the catalyst and the dimension of the metallic clusters, that govern the packing and the wrapping of the CNT bundles (Terranova *et al.*, 2004). Some representative examples of these more exotic 2D nanostructures are shown in Fig. 3.



**Fig. 3.** An example of helicoidal structures produced by the wounding of single-walled CNT. bAdapted from Ref. (Terranova et al. 2004).

In Fig. 4 FE-SEM images of surfaces covered by graphitic dendrimers are shown. These structures are very promising for the selective attachment of targeting groups.



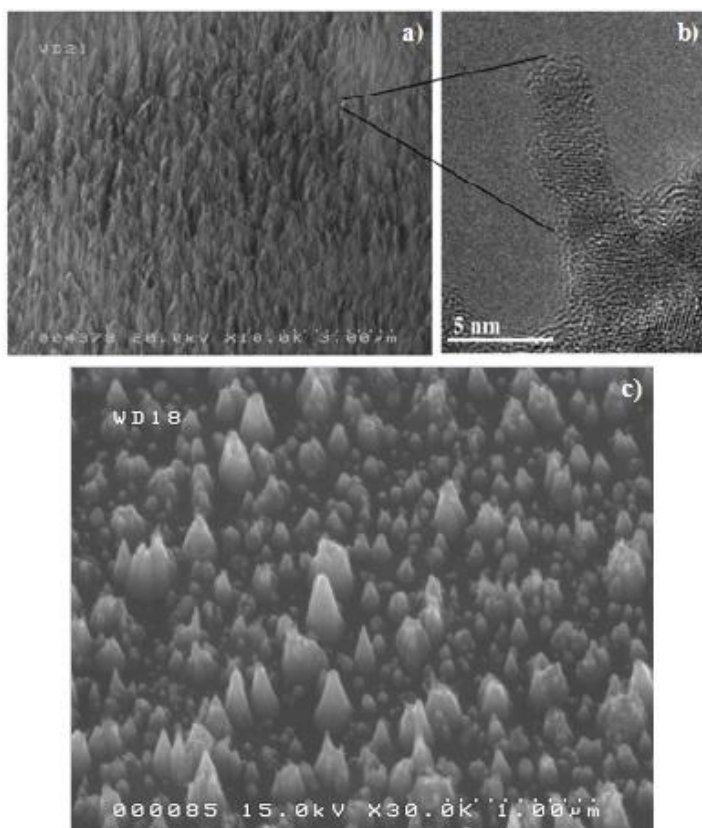
**Fig. 4.** SEM images of graphitic dendrimers deposits (unpublished before).

### **Sp<sup>3</sup> Nanocarbons**

It has been demonstrated that nanosized diamond is an excellent substrate for in vitro tissue growth (Bacakova *et al.*, 2011). The engineering of diamond at the nanoscale can be achieved either by plasma sculpturing of plane diamond deposits and isolated grains, or by the self-assembly of nanodiamond powders into a shaped structure using a solution phase methods. These two different approaches are both used in our labs for a nanometric control of the

surface topography and fabrication of low-dimensional diamond materials into desired geometries.

The first method exploits a top-down approach that can yield a variety of nanostructures by means of room temperature plasma induced H etching of plane crystalline diamond films by using a Microwave-Radiofrequency Chemical Vapor Deposition reactor. A description of the reactor specifically designed for H-etching is reported in (Toschi *et al.*, 2012) and a discussion on the mechanism that leads to the shaping of solid carbon phases can be found in (Gay *et al.* 2016). This methodology offers the feasibility to fabricate arrays of 2D elongated nanostructures, such as nanorods, nanowhiskers, nanopillars and nanocones (Orlanducci *et al.* 2012). In Fig. 5 are shown some examples of shaped nanodiamonds obtained by means of various treatments.



**Fig. 5.** a) FE-SEM images of diamond whiskers produced by plasma-etching of self-assembled detonation nanodiamonds; b) HRTEM images of an isolated whisker. Adapted from Ref. (Orlanducci *et al.* 2012). c) FE-SEM images of nanocones obtained by the etching of diamond films (unpublished before).



On CVD-produced isolated diamond crystallites (size in the range 2-3  $\mu\text{m}$ ), the same methodology allows to produce nanosized leaf-like structures that protrude from the diamond core. In Fig. 6 is illustrated an example of these rather exotic forms derived from the etching of pre-deposited large diamond grains.



**Fig. 6.** FE-SEM images of leaf-like structures obtained by plasma etching of isolated diamond grains. Adapted from Ref. (Orlanducci *et al.* 2012).

It is to be noted that, depending on the nature of the applied plasma, the surfaces of such shaped nanodiamonds can be H-, O- or OH- terminated and moreover selectively functionalized by proper after-etching chemical processes (Shenderova *et al.* 2012). A further versatility is thus offered by such engineered elongated nanostructures, that can be viewed as efficient architectures also for imaging/labelling and bio-active applications (Perevedentseva *et al.* 2013; Rosenholm *et al.* 2015).

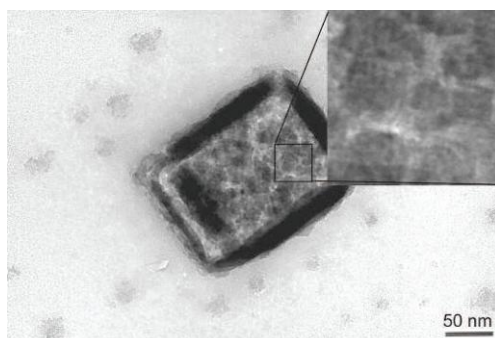
The bottom-up approach utilizes a multiscale fabrication process that leads to self-standing aggregates with pre-definite shapes. In this case the starting material is a highly stable and biocompatible nanodiamond phase generated by detonation of carbon precursors in an inert medium, and formed by polyhedral grains with sizes in the range 5-10 nm. The structure of a single crystallite of detonation nanodiamond (DND) consists of a  $\text{sp}^3\text{-C}$  core surrounded by a thin  $\text{sp}^2\text{-C}$  shell (Shugalei *et al.* 2013). The unique structural properties of DND are coupled with the same outstanding mechanical, chemical, physical properties of bulk diamond. The rich chemistry of the DND facets open the way to an easy functionalization and adduct formation, and moreover the functionalities can be further expanded by the coupling with other nanostructures (Schmidlin *et al.* 2012).

Specific physico-chemical protocols are required in order to disperse the purified nanodiamond powders (Ozawa *et al.* 2007). The treatments produce a series of different colloidal dispersions by size fractionation of various typologies of DND particles.



**Fig. 7.** Dispersions of DND in water at different concentrations (unpublished before).

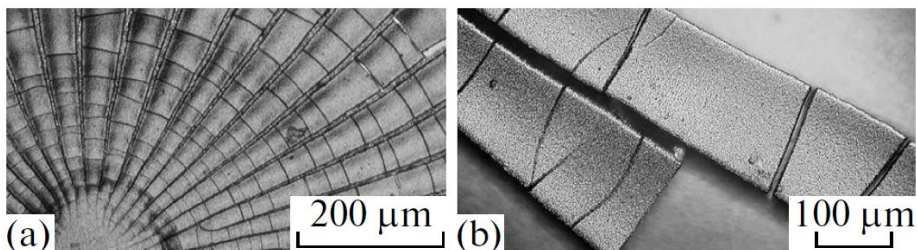
A solution-phase method based on a proper choice of solvents and drying processes enables to obtain nanosized self-assembling of diamond nanograins (Fig. 8) that maintain the same crystallographic features of diamond (s.g: Fd3m). These structures represent effective building blocks for the assembling of three-dimensional miniaturized scaffolds and platforms.



**Fig. 8.** HR-TEM image of a typical nanoassembly (sizes of about 150 x 200 nm) produced by drying colloidal stable dispersion of nanodiamond particles. The inset shows at a higher magnification the close-packed arrangement of the nanocrystals. Adapted from Ref. (Terranova *et al.* 2009).



The resulting dried materials are micro-sized solids with well-defined shapes (Fig. 9), such as slices from star-like deposits or segments from polyhedral forms (Terranova et al. 2008).



**Fig. 9.** Optical images of DND deposits: a) larger view; b) a detail of the DND units.  
Adapted from Ref. (Terranova et al. 2008).

The surface roughness of such nanodiamond-based aggregates is within 20-30 nm, a value considered very suitable for cell cultures (Bacakova *et al.* 2014).

## 2. CONCLUSIONS

In this review, it is demonstrated how the nanostructuring of  $sp^3$  and  $sp^2$  carbons and their packing in organized networks results in the forming of highly shaped local microenvironment providing a close imitation of the natural 3D extracellular matrices. Due to the inherent biocompatibility of the  $sp^2$ - and  $sp^3$ -hybridized carbon materials, the capability of shaping and tailoring the carbon structures at the nanoscale is at present a very stringent requirement for the engineering of advanced scaffolds, able to support cells and to help the development of cell functions. Moreover, proper functionalization of the nanoscale carbon structures opens the way to the fabrication of platforms for drug delivery and of innovative bio-devices.

The know-how acquired during the previous research work carried out in our labs represents now a fundamental guide-line for the preparation of bio-interfaces and bio-related functional systems.

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## CATALYTIC TESTING OF A SOLID BASE NANOMATERIAL CATALYST FROM FLY ASH

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### ABSTRACT

Fly ash is a coal combustion product and it is composed of fine particles. Millions tons per year of fly ash is produced worldwide. Fly ash can be used in concrete production, as a substitute material for cement and sand. It consists of silica, alumina, iron oxide, lime, magnesia and alkali in varying amount with some activated carbon. It possesses large surface area making it suitable for application in catalysis as well. In the present work, fly ash was chemically activated by means of alkali activation process of aluminosilicates material and further tested on the reaction of benzaldehyde with cyclohexanone. Once the alkali solution comes into contact with the raw material, the Si–O–Si, Si–O–Al and Al–O–Al bonds break to release silicon and aluminum ion into solution, which form afterwards Si–OH and Al–OH groups. The solid base catalysts produced by alkali activation of fly ash are nanocrystallites materials making them suitable not only in catalysis application but in nanotechnology as well. FTIR spectroscopy was applied to characterize fly ash before and after chemical activation. The FTIR spectrum of solid base fly ash (SBFA) after chemical activation shows a significant increase in peak intensity of the band for –OH group. GC-FID and TLC measurements showed presence of product formation for the reaction of benzaldehyde with cyclohexanone.

**Keywords:** fly ash, chemical activation, catalyst, GC-FID, FTIR spectroscopy.

### 1. INTRODUCTION

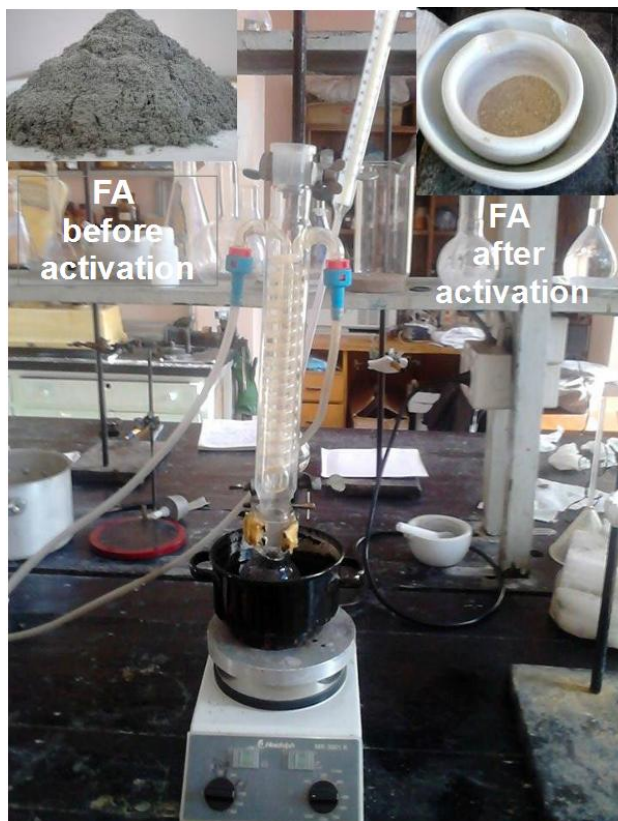
Solid base heterogeneous catalysis is an important area of industrial interest mostly in fine chemical, petrochemical and pharmaceutical industries (Andoni, 2009; Jain *et al.*, 2011). Additionally, heterogeneous catalysis is a successful application in nanoscience and nanotechnology (Andoni,

2009). Base catalyzed reactions are mostly carried out by using bases such as NaOH and  $\text{Ca}(\text{OH})_2$  (Corma *et al.*, 2005; Abello *et al.*, 2008). These bases have severe environmental issues which are associated with baseneutralization and corrosion. These issues have motivated substantial efforts to the growth of processes which apply heterogeneous catalysis. Solid bases are of high interest owing to the application of low temperature operation, and their environment friendly nature (Jain *et al.*, 2011). Fly ash is a byproduct of coal burning power plants, produced approximately 420 million tons per year globally (Jain *et al.*, 2011). It consists of silica, alumina, iron oxide, magnesia and activated carbon (Keka *et al.* 2001; Babajide *et al.*, 2010; Jain *et al.*, 2011). Moreover, fly ash possesses large surface area making it suitable for application in catalysis such as  $\text{H}_2$  production,  $\text{deSO}_x$  and  $\text{deNO}_x$  (Wang, 2008). Jain *et al.*, (2011) reported that fly ash can be chemically activated by NaOH solution giving though the surface basicity and making it catalytically active. Additionally, F-type fly ash, rich in silica and alumina content with sufficient  $-\text{OH}$  groups, was utilized to catalyze organic chemical reactions (Jain *et al.*, 2011). In the current work fly ash was chemically activated by an alkali activation process of aluminosilicates material in resemblance to the work of Jain *et al.*, 2011. The aim is to successfully activate fly ash and further test it as a catalyst in the reaction of benzaldehyde with cyclohexanone. This reaction has large application in fine chemical industries. FTIR spectroscopy was applied to characterize fly ash before and after chemical activation. IR spectra clearly indicated an augment in the peak intensity of the band for  $-\text{OH}$  group after chemical activation for the fly ash. GC-FID was applied to analyze the reaction mixture.

## 2. MATERIAL AND METHODS

**Sample preparation:** The solid fly ash was provided by a coal burning power plant, F-type fly ash, rich in silica and alumina content, Gjiyli, 2011. The solid base fly ash catalyst (SBFA) was synthesized by chemical activation of fly ash with NaOH (98%, Merck). Concentration of alkali solution used was 50 wt.% (Jain *et al.*, 2011; Andoni *et al.* 2015). The chemical activation was carried out in a round flask (equipped with a stirring bar and a condenser, Figure 1) by taking the mixtures of fly ash and NaOH solution, followed by subsequent heating at  $110^\circ\text{C}$  under stirring and aging for 2 days preserving the temperature. After ageing, the obtained pulp was washed with distilled water to remove leached compounds and excess of NaOH. The pulp was dried at  $110^\circ\text{C}$  for 24 h. The chemically activated fly ash was afterwards thermally stabilized by calcination at  $450^\circ\text{C}$  for 4 h in static conditions. FTIR

spectra were collected by Nicolet 6700 spectrometer, manufactured by Thermo Electron.



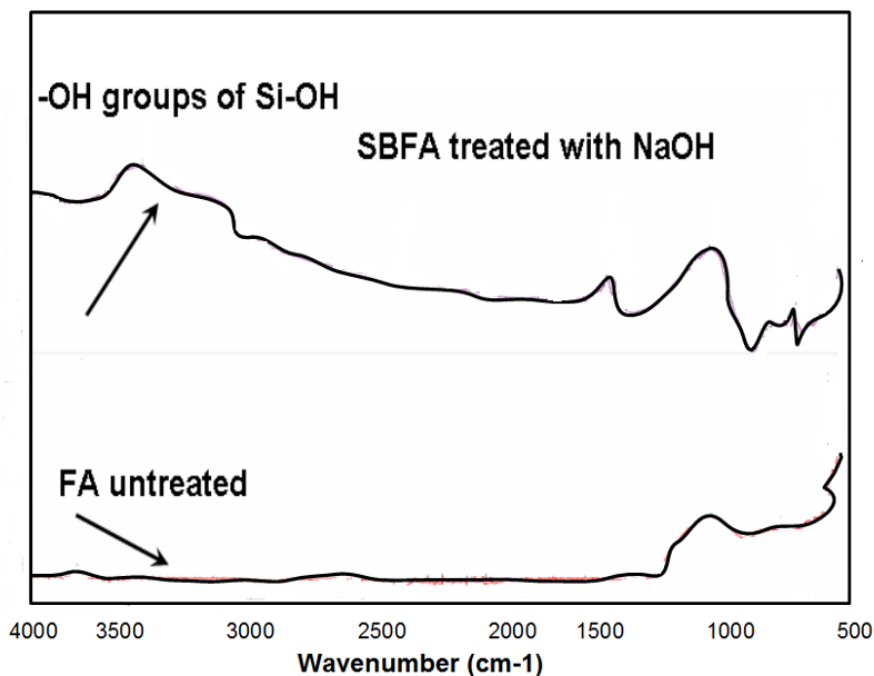
**Fig. 1:** Photo of the set up for the chemical activation of the fly ash. In the inserts additional photos of the fly ash before and after chemical activation are presented.

The condensation of cyclohexanone(98%, Merck) and benzaldehyde (98%, Merck) using SBFA was carried out in a liquid phase reactor following though the procedure reported by Jain *et al.* 2011. Cyclohexanone and benzaldehyde (molar ratio of cyclohexanone and benzaldehyde = 1:2) were taken in a 50 ml round bottom flask, equipped with magnetic stirrer and condenser, immersed in a constant temperature oil bath. The catalyst (benzaldehyde to catalyst weight ratio = 10:1) was activated at 450<sup>0</sup>C for 2 h prior to the reaction in static air, and then added in the reaction mixture. The reaction mixture was heated at required reaction temperature ranging from 90 to 140<sup>0</sup>C and time from 30 min to 4 h at atmospheric pressure. After the completion, the reaction mixture was cooled and filtered to separate the catalyst and was analyzed by

gas chromatograph (GC HP 5890 series 2) having a flame ionization detector and HP-5 capillary column and  $N_2$  as a carrier gas.

### 3. RESULTS AND DISCUSSION

The FTIR spectrum of fly ash (FA), before and after chemical activation is shown in Figure 2. Fly ash was chemically activated with 50 wt.% alkali solution (Jain *et al.* 2011, Andoni *et al.* 2015). The FTIR spectra of solid base fly ash (SBFA) shows a broad band in the region between  $3400-3000\text{ cm}^{-1}$ , which is attributed to surface  $-OH$  groups of  $-Si-OH$  (Jain *et al.* 2011). The broadness of the band is due to the strong hydrogen bonding (Smith, 1999). The hydroxyl groups are not isolated and hence a high degree of association is experienced as a result of extensive hydrogen bonding with other hydroxyl groups (Smith, 1999; Jain *et al.*, 2011).



**Fig.2:** Wide scan FTIR spectrum fly ash (FA) before and after treatment with NaOH (NaOH concentration, 50 wt.%).

It is relevant to mention that water which absorbs on the surface gives also a broad band in the region between  $3400-3000\text{ cm}^{-1}$ , however, FTIR spectrum of untreated FA displays a flat line in the region  $3400-3000\text{ cm}^{-1}$ . This



suggests that the broad band in the FTIR spectrum of SBFA (after chemical activation) in the region  $3400\text{--}3000\text{ cm}^{-1}$  belongs to the  $\text{--OH}$  groups of  $\text{--Si--OH}$  and not to the  $\text{--OH}$  of water molecules. Furthermore, water gives a characteristic peak at  $1650\text{--}1630\text{ cm}^{-1}$  in the IR spectrum, which is attributed to bending mode ( $\delta_{\text{O--H}}$ ) of water molecule (Jain *et al.* 2011 and Smith, 1999). In this respect, both FTIR spectra before after chemical activation of fly ash (Figure 2) show no peaks in the region of  $1650\text{--}1630\text{ cm}^{-1}$ . Nevertheless, minor amount of water absorbed on the surface of FA or SBFA cannot be excluded.

Additionally, IR analysis of our former work (Andoni *et al.*, 2015) indicated an increase in the peak intensity of the band for the  $\text{--OH}$  groups upon increasing the concentration of NaOH incrementally. This was, however, more evident in the peaks intensities appearing in the range  $1000\text{--}950\text{ cm}^{-1}$  (this is apparent also in Figure 2), which were attributed to the Si-O-Si and Si-O-Al asymmetric stretching (Andoni *et al.*, 2015). Upon increasing the concentration of alkali solution, the peak position for the Si-O-Si and Si-O-Al asymmetric stretching moved to lower wavenumbers. The peak shifting was explained due to the increased population of  $\text{--OH}$  groups on the surface (Jain *et al.* 2011, Smith, 1999, Andoni *et al.* 2015). Additionally, Jain *et al.* 2011 reported that fly ash activated by alkali solution had significantly increased crystalline phase. The crystallite size of solid base fly ash (SBFA) was reported to be 11 nm (Jain *et al.*, 2011). Gilyly (2011) also reported presence of crystalline phase (SEM images) for activated fly ash by different methods. A preliminary test on SEM imaging (Figure 2) indicated crystalline and porous structure for fly ash activated by alkali solution in agreement with the work of Jain *et al.* 2011 and Gilyly, 2011.

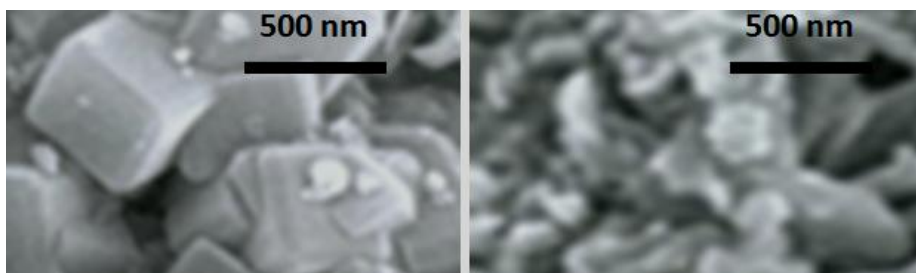
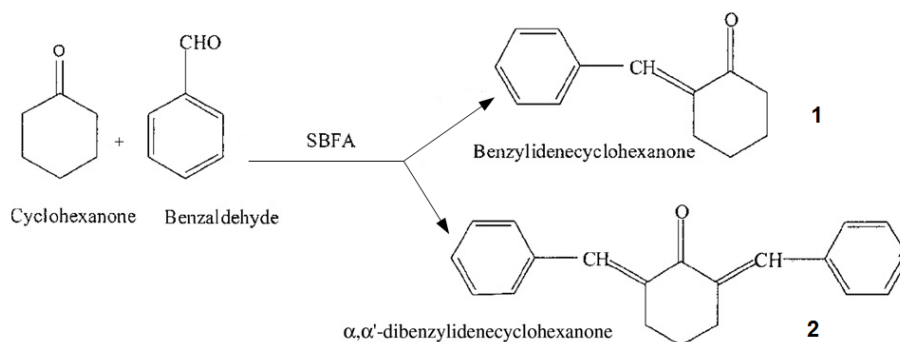


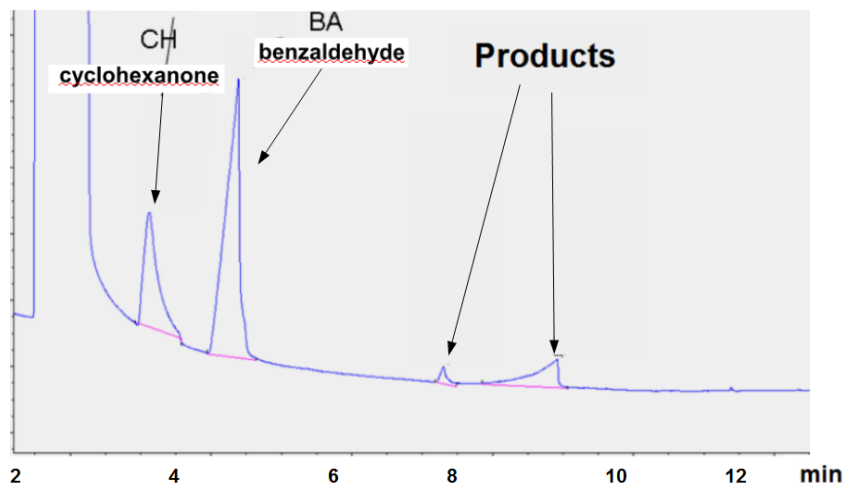
Fig. 2: SEM images of fly ash activated by alkali solution.

To this end, the activated fly ash was tested as a catalyst. The reaction of benzaldehyde with cyclohexanone (Claisen–Schmidt condensation, scheme 1, Jain *et al.* 2011), was allowed to carry out on the SBFA (concentration of alkali solution, 50 wt.%). Jain *et al.* 2011, reported high conversion and high selectivity to product **2** in the scheme 1.



**Scheme 1.** The Claisen–Schmidt condensation of cyclohexanone and benzaldehyde using SBFA catalyst.

Figure 4 displays GC chromatogram of the reaction mixture for the Claisen–Schmidt condensation. It is evident from GC chromatogram that the reaction mixture gives rise to products formation. GC chromatogram indicates presence of two products. GC results were supported by thin layer chromatography (TLC, not shown). However, presence of unreacted cyclohexanone (CH) and benzaldehyde (BA) is also obvious in the GC chromatogram. The presence of the reactants may be explained by insufficient basic sites both on the surface and in the bulk, responsible for the catalytic activity. Sufficient surface basicity in SBFA promotes the condensation of cyclohexanone to benzaldehyde.



**Fig. 4:** GC chromatogram of the reaction mixture for the Claisen–Schmidt condensation.

#### 4. CONCLUSION

In the current work fly ash was used as a heterogeneous catalyst. It was activated by alkali solution and characterized by FTIR spectroscopy. FTIR spectra featured similarities to a large extent with those reported in the literature (Jain *et al.* 2011). A preliminary test of the Claisen–Schmidt condensation, i.e. reaction of benzaldehyde with cyclohexanone carried out on the surface of the solid base fly ash indicated product formation. However, GC indicated presence of cyclohexanone (CH) and benzaldehyde (BA) as well. The partial conversion to product formation may indicate the lack of sufficient basic sites both on the surface and in the bulk. Surface basicity in SBFA promotes the condensation of cyclohexanone to benzaldehyde. Therefore, increased surface –OH groups (surface –OH groups of –Al–OH and Si–OH) by increasing alkali concentration might give higher conversion of reactants to products.

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## USE OF NANOMATERIALS IN AGRICULTURE AND ENVIRONMENT: CURRENT STATUS IN ALBANIA

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### ABSTRACT

Nanomaterials like zeolites, clays, biochar, etc., have a large number of applications in agriculture and food production, medicine and environment. Use of nanomaterials in agriculture and environment aims to reduce application rates of pesticides, minimize nutrient losses due to leaching and increase plant productivity and protect the environment. This paper present some experimental data in terms of improving chemical characteristics of soils, reducing plant uptake of heavy metals in the contaminated soils and reducing of nutrient or nitrate leaching. Obviously positive effects of zeolite on soil chemical properties and plant growth as well as on reduction of availability and plant uptake of heavy metals are observed. Also, the data showed a decrease of nitrate leaching in soil by application of biochar. *However, no data are available* for the effects of zeolite and other nanomaterials on mobility of nutrients in soil and *improvement of fertilizer use efficiency*. Therefore, further studies have to be performed related to their use for agriculture and environmental purposes.

**Key words:** natural zeolite, biochar, soil, nitrate leaching, nutrient uptake.

### 1. INTRODUCTION

Nanomaterials are present in different geological formations and include minerals like clay, micas, zeolites and iron oxides/hydroxides (Petr O. Vasiliev, 2009). The zeolites have emerged as having considerable potential in a wide variety of uses, including agronomy and horticulture (Mumpton, 1999a). The use of zeolites in combination with other soil amendments (organic and inorganic fertilizers) was reported to improve the soil properties and increase the yields of plants (Allen and Ming, 1995; Mumpton, 1999b; Stead et al., 2002; Beqiraj Goga et al. (2008); Glisic et al., 2009). However, little is known about the effect of zeolite use on yield and nutrition of plants in

the problem soils (clay, sandy, saline, and sodic soils), and on mobility of heavy metals in the contaminated soil.

Nitrate leaching may be a major problem in certain soils after intensive use of nitrogen fertilizers (Cepuder et al., 2008). Moderate use of organic amendments, i.e. manure, peat, sewage sludge and biochar, can supply available N and reduce nitrate leaching (Antoniadis et al., 2007; Brahushi et al., 2000; Laird et al., 2010; Shepherd and Newell-Price, 2013; Giola et al., 2012; Ploechl et al., 2016). Other studies have indicated that application of organic amendments decreased N losses to the environment most likely due to increased plant N uptake, microbial N immobilization, and consequent reduction in nitrification, denitrification, and nitrate leaching (Vano et al., 2011, Yao et al., 2012). This paper summarizes and *discusses the* results of several studies on the effect of natural zeolites on chemical properties and performance of ryegrass grown in a sandy soil and on heavy metals transfer from contaminated soil to plant, as well as on the effect of biochar on nitrate leaching from a loamy soil.

## **2. Use of natural zeolites as an amendment for sandy soils**

### ***2.1 Effect of zeolites on soil chemical properties***

An experiment was carried out at the Agricultural University of Tirana (2005) in a randomized block design with seven treatments replicated four times. The treatments consist of control, NPK, 6 q zeolite ha<sup>-1</sup>, 12 q zeolite ha<sup>-1</sup>, 6 q zeolite ha<sup>-1</sup>+NPK, 12 q zeolite ha<sup>-1</sup>+NPK, zeolite at 5 % (w/w basis). A recommended dose of NPK at 148, 75 and 60 kg ha<sup>-1</sup> active substance was applied to each pot. Natural Stilbite-Stellerite from Munella region (Albania) and ryegrass plant were used in this study. *Chemical composition* of the studied zeolite is described by Beqiraj Goga et al. (2008). The zeolitic material was ground and sieved to 100 mesh size.

All treatments decreased soil fertility parameters, except CEC where, however, only use of zeolite at 5% by soil weight had significant effect (Table 1). Significant lower content was observed for N in NPK, 6 q zeolite ha<sup>-1</sup>, 12 q zeolite ha<sup>-1</sup> treatments; humus, P and K in all treatments; and Cu in NPK, 6 q zeolite ha<sup>-1</sup>+NPK, 5% zeolite treatments, Fe in 5% zeolite treatment, Zn and Ca in all treatments; and higher CEC value in 5% zeolite treatment due to direct effect of zeolite on nitrogen capture, storage and release (Akinyemi, 2006), mineralization of biogenic substances by microorganisms (Antinis et al., 2002.), solubilisation of phosphate minerals (Chesworth et al., 1987; Lai and Erbel, 1986) and phosphorous uptake by plants (Barbarick et al., 1990), and on soil CEC.

**Table 1.** Soil chemical properties at studied treatments before (a) and after (b) experiment (Gjoka et al., 2011)

Soil property	T <sub>1</sub> Control		T <sub>2</sub> NPK		T <sub>3</sub> Zeolite 6 q ha <sup>-1</sup>		T <sub>4</sub> Zeolite 12 q ha <sup>-1</sup>		T <sub>5</sub> Zeolite 6 q ha <sup>-1</sup> + NPK		T <sub>6</sub> Zeolite 12 q ha <sup>-1</sup> + NPK		T <sub>7</sub> Zeolite at 5%		LSD
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Humus (%)	0.8	0.7*	0.8	0.6*	0.8	0.6*	0.8	0.6*	0.8	0.7*	0.8	0.7*	0.8	0.7*	(p<0 .05)
N-total (%)	0.07	0.06	0.07	0.05*	0.07	0.05*	0.07	0.05*	0.07	0.06	0.07	0.06	0.07	0.06	0.01
Available P (mg/100 g)	1.32	0.77*	1.32	0.81*	1.32	0.67*	1.32	0.92*	1.32	0.95*	1.32	0.85*	1.32	0.21*	0.24
Exchange K (mg/100)	7.5	4.33*	7.5	4.83*	7.5	4.00*	7.5	4.83*	7.5	4.17*	7.5	4.22*	7.5	4.31*	0.31
CEC (me/100 g)	11.5	10.0	11.5	13.4*	11.5	11.8	11.5	12.7	11.5	12.8	11.5	10.7	11.5	9.6*	1.44
CaCO <sub>3</sub> (%)	7.14	8.1*	7.14	7.5	7.14	8.0*	7.14	7.3	7.14	6.9	7.14	7.4	7.14	7.4	0.40
Total-Cu (mg kg <sup>-1</sup> )	44	40.0	44	35.2*	44	41.3	44	42.6	44	28.8*	44	40.4	44	25.6*	6.46
Total-Zn (mg kg <sup>-1</sup> )	78	63.2*	78	46.0*	78	52.3*	78	50.3*	78	49.2*	78	51.3*	78	49.2*	5.30
Total-Mn (%)	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.01
Total-Fe (%)	4.6	4.67	4.6	5.37*	4.6	4.57	4.6	5.08	4.6	5.37*	4.6	3.98	4.6	3.08*	0.73
Total-Ca (%)	8.8	4.36*	8.8	2.93*	8.8	5.33*	8.8	4.31*	8.8	3.04*	8.8	4.38*	8.8	3.42*	0.84

The lowest values of N (0.05%), Zn (46.0 mg kg<sup>-1</sup>), Mn (0.06 mg kg<sup>-1</sup>), and Ca (2.93 mg kg<sup>-1</sup>), were recorded in NPK treatment, Cu (25.6 mg kg<sup>-1</sup>) and Fe (3.08 mg kg<sup>-1</sup>) in 5% zeolite treatment, P (0.67mg 100g<sup>-1</sup>) and K (4.00 mg 100g<sup>-1</sup>) in 6 q zeolite ha<sup>-1</sup> treatment. The highest value of CEC (15.55 me 100 g<sup>-1</sup>) was noted in 5% zeolite treatment. Effect of zeolite on CEC could be related to its mineralogical composition. These results suggest that zeolitic rocks from Munella region may be a potential source of amendment for use to sandy soil in improving soil chemical properties. Zeolite at 6 q ha<sup>-1</sup>+NPK was most significant in maintaining and improving soil properties and nutrient status in soil.

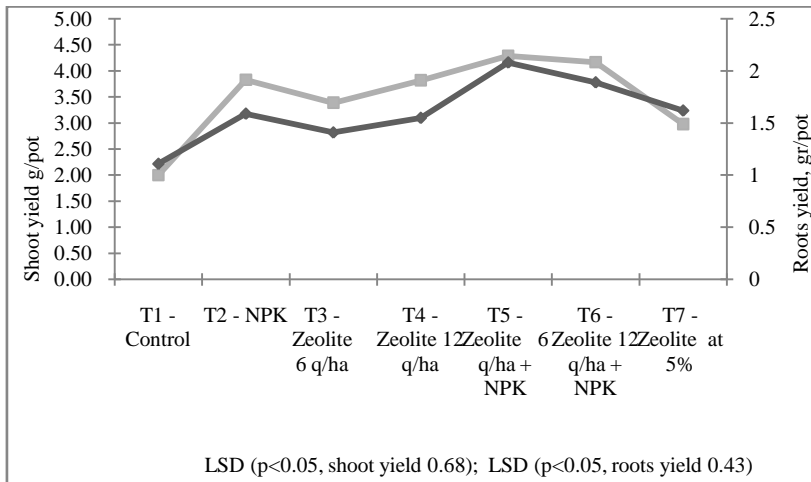
## 2.2. Effect of zeolites on plant growth and nutrient uptake in ryegrass

The above-mentioned experiment was also used to study the effect of natural zeolite and NPK on the growth and nutrient status of ryegrass (*Lolium multiflorum* L.). Use of zeolite and NPK resulted in a significantly increase in the shoot and roots yield of ryegrass compared to control. The highest shoot yield (4.29 g pot<sup>-1</sup>) was recorded in the treatment receiving 6 q zeolite ha<sup>-1</sup>+NPK. Zeolite applied directly (12 q ha<sup>-1</sup> and 5 % of soil weight) or together with NPK increased significantly the yield of roots over control, but not over NPK except application of 6 q zeolite ha<sup>-1</sup>+NPK giving the highest root yield (2.08 g pot<sup>-1</sup>) (Figure 1).

Zeolite leads to a more efficient use of NK fertilizers by reducing their rates for the same yield, by prolonging their activity or finally, by producing higher yields (Polat, et al. 2004). Substantial increases were noticed in the content and uptake of nutrients in shoot due to application of zeolite and NPK. Zeolite at 6 q ha<sup>-1</sup>, 12 q ha<sup>-1</sup> and 5% and zeolite at 12 q ha<sup>-1</sup>+NPK recorded the highest P, K, Fe and Zn content and uptake of these nutrients of ryegrass shoot (Figure 2 and Figure 3). The highest content of Cu and Mn in

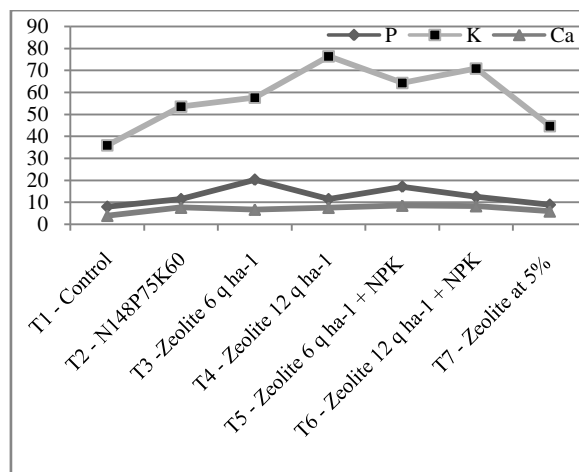
shoot was observed in control and this may be due to the rate of selectivity for cations  $\text{Zn}^{2+} < \text{Cu}^{2+} < \text{Pb}^{2+}$  of the zeolite used (Beqiraj Goga et al., 2008)). Also, zeolites display different affinities for cations and form the theoretical basis of their cation selectivity sequence (Pansini, 1996).

The highest content of Mn in control may be due to high binding capacity of Fe-Mn oxides and aluminosilicates for metals. These effects were assumed to be related to immobilization of metals due to formation of insoluble metal-organic complexes and increased CEC (Singh, 2001). It could be concluded that the tested zeolite functioned as a good regulator of plant nutrition in sandy soil amended with NPK fertilizers. Use of natural zeolite at  $6 \text{ q ha}^{-1}$  together with NPK fertilizers at  $148:75:60 \text{ kg ha}^{-1}$  was the best treatment in increasing dry matter yield of ryegrass and in enhancing the uptake of nutrients by ryegrass.

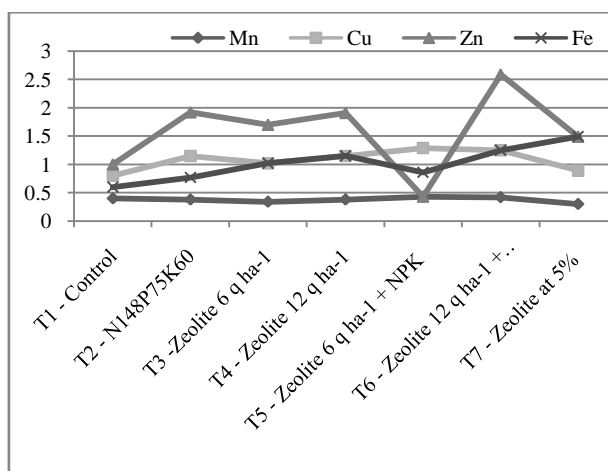


**Figure 1.** Ryegrass shoot and roots yield (Gjoka et al., 2011)





**Figure 2.** P, K and Ca uptake by ryegrass (mg pot<sup>-1</sup>) (Gjoka et al., 2011)



**Figure 3.** Mn, Cu, Zn, Fe uptake by ryegrass (mg pot<sup>-1</sup>) (Gjoka et al., 2011)

### 3. Use of zeolites to immobilize heavy metals in contaminated soils

A pot experiment using a soil contaminated with heavy metals from former smelting area (Rubik) and five rates of 100-mesh natural zeolite powder (0, 1.25, 2.5, 5, and 10%, w/w) was performed at the Agricultural University of Tirana in 2014. *Chemical composition of the tested zeolite was as follows CEC 6.72 meq/100 gr, pH H<sub>2</sub>O 8.7, EC (1:5) 74 μS/cm* (F. Gjoka, personal communication). The results indicate that the DTPA-extractable Ni, Cu, Zn and Cd contents decreased significantly by adding the zeolite to

polluted soil at the rate 10%, while the leachability of Cu, Cd, Ni and Zn *apparently* reduced by adding the zeolite at the rate 2.5% (unpublished data). The pattern of decreasing relative metal contents in amended soils was not consistent, but generally similar for all metals. The chemical analysis results of the plant samples indicate that the applied zeolite at the rate 5% led to decreased heavy metal contents (Ni, Cd, Cu, Zn) in ryegrass plant, especially in the first cutting in comparison to non-amended soil (unpublished data). Application of zeolite at the rate 10% decreased notably the contents of Ni, Zn, Cu, and Cd in shoots and of Zn and Cu in roots (unpublished data).

#### 4. Impact of biochar on nitrate leaching in soil

In order to evaluate impact of biochar as soil amendment on nitrate leaching, an experiment was performed at the Agricultural University of Tirana during the year 2015, in a design with four treatments and replications. The treatments were as follow: V1- Soil without any amendments and no planted; V2- Soil without any amendments and planted; V3- Soil + NPK fertilizers and planted; and V4- Soil + Biochar+ NPK fertilizers and planted. An amount of 2 kg sieved soil (<2mm) was used in each pot. The main soil characteristics are presented in the Table 2. Wheat straw biochar as soil amendment was applied to the rate 10 g/kg soil. Nono-structure, physical and chemical properties of biochar are well described by Joseph et al., 2013. After planting with ryegrass plant (*Lolium multiflorum* L.), the soils were brought to soil WHC by adding distilled water. NPK (15:15:15) fertilizer in total amount of 1.665 g/kg soil was applied 3 times during the vegetation, thus 1<sup>st</sup> treatment was 2 weeks after sowing, 2<sup>nd</sup> treatment 1 week after first cutting, and 3<sup>rd</sup> treatment 1 week after second cutting. The amount of N-NO<sub>3</sub> was measured in leachate after each cutting and collecting of green part of the plant.

**Table 2.** The main soil properties

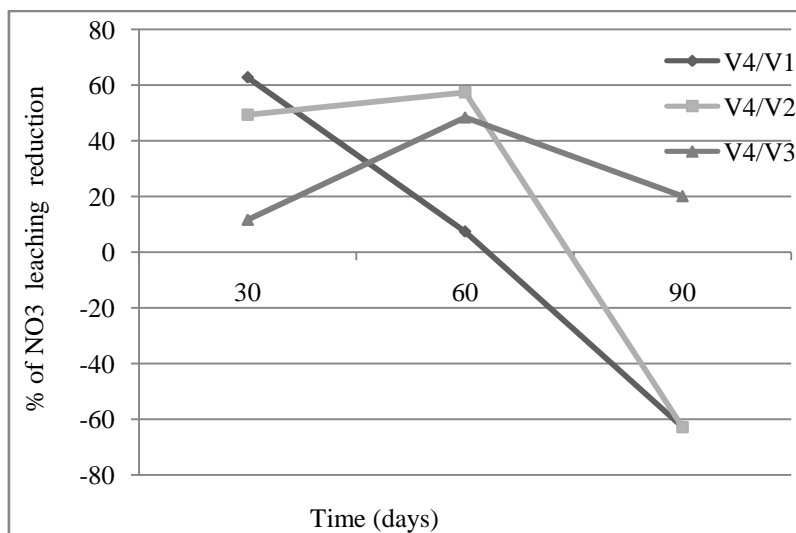
Physical characteristics				Chemical characteristics		
pH	(%)			g/kg soil d.w. (dry weight)		
8	Sandy	Silt	Clay	N-total	P-total	C -organic
	43.18	33.67	23.14	2.132	1.051	19.474

The obtained results on reduction potential of nitrate leaching in soil amended with biochar compare to the other treatments are presented in the Table 3. These data show that the use of biochar as soil amendment clearly reduced nitrate leaching from soil due to its adsorption capacity. Thus, after

first cutting of ryegrass plants the measured amount of  $\text{N-NO}_3$  in treatment with biochar was reduced from 12 to 63% compare to the other treatments. Thus, the use of biochar can reduce groundwater pollution with nitrate. Furthermore, obtained data showed that during the vegetation time the potential of biochar to reduce nitrate leaching was reduced (see Figure 4), showing limiting effect of biochar for long time.

**Table 3. Reduction of  $\text{N-NO}_3^-$  leaching in soil**

Treatments rapport		% of $\text{NO}_3$ Leaching reduction		
		First Cut	Second Cut	Third Cut
	V4/V1 (%)	62.82	7.39	-62.85
	V4/V2 (%)	49.38	57.41	-62.85
	V4/V3 (%)	11.63	48.40	20.12



**Figure 4.** Time course of biochar potential to reduce nitrate leaching form soil

## 5. CONCLUSIONS

The uses of nanomaterials like natural zeolites, biochar, clays, etc., in agriculture and environment are still limited in Albania, although these materials are usually not very costly and these study results are very encouraging. Therefore, more systematic and in-depth researches on the zeolite and biochar for their chemical composition and agricultural and

environmental uses are needed. In addition, there is the possibility for the pre-treatment of these materials to improve their performance in ion exchange. However, the current research works with the natural zeolite are not focused on such chemical and physical treatments. It is for this reason that is suggested the creation of a strategy to make possible the use of nanomaterials available in Albania as soil conditioner and biosensors for agricultural and environmental purposes.

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## A STATISTICAL NANOINDENTATION ASSESSMENT OF VOLUME FRACTIONS OF MAIN PHASE-CONSTITUENTS OF ULTRA-HIGH PERFORMANCE CONCRETE

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### ABSTRACT

A statistical nanoindentation assessment of the main phases, especially of the C-S-H phase of three ultra-high performance concrete (UHPC) samples which contain nanoscale pozzolan, cured with or without microwave energy, is reported. Using nanoindentation technique, a quantitative phase assessment was carried out. There is considerably more C-S-H phase and more homogeneous elemental and phase distribution in the microwave cured specimens. Compared to the non-treated samples there is about 46.5% more total C-S-H phase content in the microwave cured sample and about 40.2% more C-S-H phase in the microwave cured and pressed sample. Curing with microwave energy mainly increases the content of low density C-S-H at the expense of portlandite phase.

**Keywords:** ultra-high performance concrete, microwave treatment of UHPC, mechanical properties of cementitious materials, nanoindentation technique

### 1. INTRODUCTION

The advent of instrumented indentation provides an unprecedented opportunity to measure the mechanical response of material and its composing phases at appropriate length scales (Fischer-Cripps, 2011). The indentation technique consists of establishing contact between an indenter of known geometry and mechanical properties (typically diamond) and the indented material for which the mechanical properties are of interest. Subsequently, the continuous change in penetration depth  $h$  as a function of increasing indentation load  $P$  ( $P-h$  curve) is acquired (Oliver et al., 1992). Typically, the extraction of properties is achieved by applying a continuum

scale mechanical model to derive two quantities, indentation hardness  $H$  and indentation modulus  $E$  (Oliver et al., 2004). In particular, a novel grid indentation technique can provide both key mechanical properties of individual phases comprising the material and access to the volume fractions of independent phases (Constantinides *et al.*, 2006).

Three ultra-high performance concrete (UHPC) samples, which contain nanoscale pozzolan and have been cured with and without microwave energy (Korpa and Trettin, 2008) were examined by using nanoindentation technique, in order to better understand the reason for the early strength development and the related chemical-physical processes that continuously occur inside the hardening cementitious matrix. An assessment of the main phases and especially of the C-S-H phase composition was carried out through a statistical nanoindentation analysis, which provided access to the mechanical properties and volume fractions of all phase-constituents of the material. The quantification of the mechanical contribution of the main phases, especially the two C-S-H types, on the macroscopic material properties has been reported. There is considerably more C-S-H phase and more homogeneous elemental and phase distribution in the microwave cured specimens.

## **2. Experiments**

### **2.1. Materials**

Three cementitious pastes were prepared, using Portland cement, silica fume and fly ash as microscale pozzolanic additives, pyrogenic oxide as nanoscale pozzolanic additives and superplasticizers with polycarboxylate ether base. Table 1 shows the chemical composition of microscale ingredients of the cementitious pastes. The water to cement ratio was kept 0.2. Table 2 presents the compositions and mass ratios used for the preparation of the pastes (Korpa and Trettin, 2008).



**Table 1.** Chemical composition in wt. % of three principal ingredients of cement pastes

Components	PC I 52.5 R HS/NA	Silica fume, SF (G983)	Fly ash, FA (SWF)
SiO <sub>2</sub>	19.60	98.70	54.10
Al <sub>2</sub> O <sub>3</sub>	3.22	0.28	22.10
Fe <sub>2</sub> O <sub>3</sub>	4.90	0.10	7.71
CaO	66.90	0.23	5.11
MgO	0.87	0.11	2.98
Na <sub>2</sub> O eq.	0.73	0.30	4.22
SO <sub>3</sub>	3.12	0.19	2.28
P <sub>2</sub> O <sub>5</sub>	0.20	0.02	0.18
Others + LOI	0.46	0.07	1.32
d <sub>50</sub>	6.70 μm	0.35 μm	6.50 μm

**Table 2.** Mix proportions of ingredients and their mass percentage in the cementitious pastes

Ingredients	Cem	FA	SF	POx	SP	W
Mix proportion	1	0.17	0.24	0.03	0.02	0.2
wt %	60.24	10.24	14.46	1.81	1.20	12.05

**Cem** – PC I 52.5 R HS/NA, **FA** - fly ash (SWF), **SF** - silica fume (G983), **POx** - pyrolytic oxides (supplied from Degussa), **SP** – superplasticizer (supplied from Degussa), **W** - water

## 2.2 Sample treatment

The mixing process lasted approximately 8 minutes, during which the uniform distribution of the ingredients took place. The cementitious pastes (will be referred to as “pastes”) were casted into 1x1x4 cm steel molds, which were kept in temperature  $20 \pm 1^\circ \text{C}$  and relative humidity  $95 \pm 5\%$ . One of the samples was kept under a load of 5T by means of a laboratory press. After 24 hours, the hardened pastes were de-molded and cured according to the procedure described in detail in a previous work (Korpa and Trettin2008). The samples are denoted as follows: the basic sample, cured at temperature  $20 \pm 1^\circ \text{C}$  and  $95 \pm 5\%$  rh is denoted as “C000”; “CMW0” is denoted the sample treated thermally in a microwave oven, model MW-17, with frequency 2450 MHz and installed power of 800 W, under a program which resulted very convenient and lasted 60 minutes at 150 W, followed by 30 minutes at 250 W and 10 min at 280 W; the sample, that was kept under load

before microwave treatment took place, was denoted as “CPMW”. After 7 days of curing, before the nanoindentation tests, the samples were carefully polished to achieve a smooth surface. The samples have undergone nanoindentation tests. Two mechanical parameters, the elastic modulus and the hardness, were simultaneously obtained from the initial slope of the load-depth curve (Fischer-Cripps, 2011). A nanoindenter type Hysitron Inc (TriboIndenter) with a Berkovich tip was employed, in a matrix of 300 nanoindentations. The analysis of 300 data points per sample was done by statistical deconvolution technique.

### 3. Data processing and results

The frequency distribution analysis was conducted for the measured values of elastic modulus ( $E$ ) and hardness ( $H$ ), by choosing as bin size (group-interval) 2 GPa and 0.2 GPa for the elastic modulus and hardness respectively (Mondal *et al.*, 2010). Fig. 1 shows the distribution of frequencies for the elastic modulus and fig. 2 for the hardness. It seems that we are dealing with a typical distribution for hardened pastes, whose composition is dominated by calcium-silicate-hydrate and calcium hydroxide (portlandite)(Ulm *et al.*, 2007; Nemecek 2012).

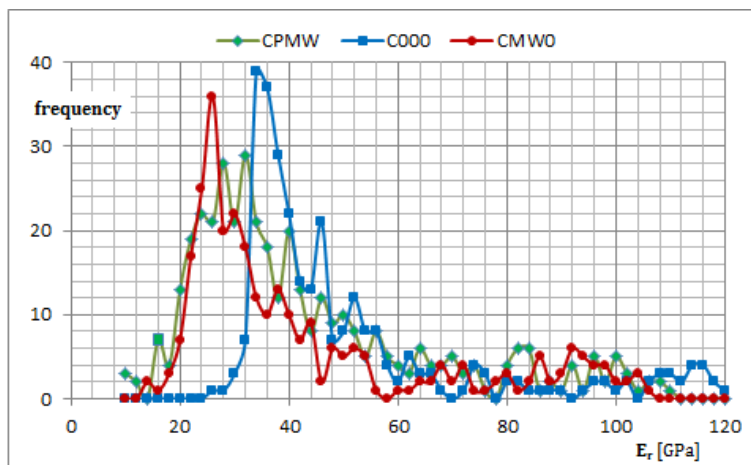


Fig. 1. Frequency distribution of indentation modulus: bin size 2 GPa

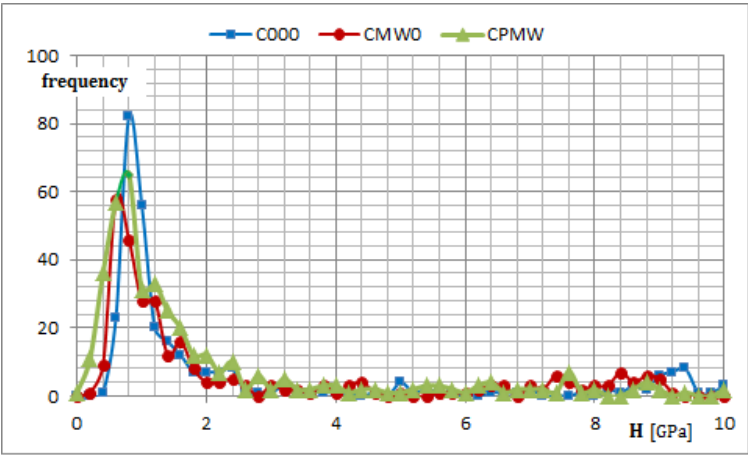


Fig. 2. Frequency distribution of indentation hardness: bin size 0.2 GPa

Before analyzing the frequency distribution plots, one must determine the intervals for the values of the elastic modulus and hardness corresponding to a certain phase-constituent in the sample. Due to the very high heterogeneity that characterizes paste ingredients and different applied cure conditions, the recommended intervals do not overlap with each other (Kaushai *et al.*, 2012). The large number of experimental data allowed, however, correlating them. Based on this correlation, the interval values for elastic modulus and hardness of six phase-constituents were determined and those values are summarized in Table 3.

**Table 3.** Interval values of E and H for the phase constituents of cementitious material

Phase constituents	M/N Porosity	CSH LD	CSH HD	CH (Portlandite)	SF/FA/Q	Clinker (anhydrous)
Interval of E (GPa)	< 15	15 - 26	26 - 36	36 - 53	53 - 92	> 92
Interval of H (GPa)	< 0.4	0.4 – 0.8	0.8 – 1.6	1.6 – 3.2	3.2 – 6.5	> 6.5

**M/N Porosity** = micro/nano-porosity, **CSH** = calcium-silicate-hydrate, **LD** = low density, **HD** = high density, **SF/FA/Q** = silica fume/fly ash/quartz (powder or sand)

For the main phase-constituents (CSH and CH), the found intervals resulted valid, even in the case of pastes containing additives as SF. This

supports the conclusions of several authors (Sorelli *et al.*, 2008; Costandines *et al.*, 2004; Mondal *et al.*, 2010; Hu and Li 2015) that the presence of pozzolanic additives will not affect the interval values of  $E$  and  $H$  but the volume fraction of phase-constituents.

Based on elastic modulus, Fig. 3 and 4 illustrates respectively the frequency distribution and phase volumetric percentage for three main phase-constituents of the samples.

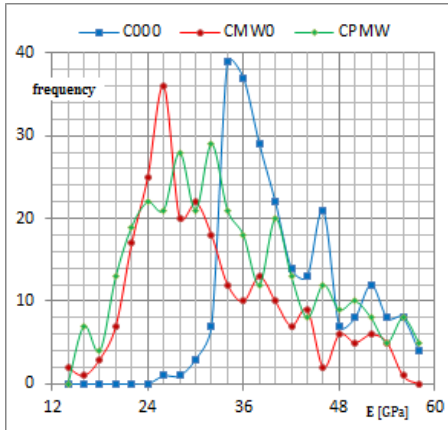


Fig. 3. Frequency distribution of indentation modulus for C-S-H-s and CH

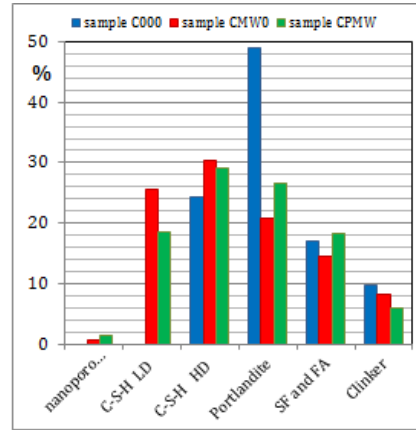


Fig. 4. Histograms of phase volumetric percentage based on elastic modulus

The volume fraction of each phase-constituent has been evaluated by taking into account the thresholds characterizing the phase-constituents (Table 3). Table 4 summarizes the volume fractions obtained from the frequency distribution of indentation elastic modulus, for each of six phase-constituents and for the three main phase-constituents of the samples.

**Table 4.** Volume percent fractions of phase-constituents in the hardened pastes.

Sample	Nano-porosity	CSH low density	CSH high density	CH	SF & FA	Clinker	CSH LD + CSH HD	CSH LD + CSH HD + CH
C000	0.00	1.02	29.5	42.7	16.9	9.83	30.5	73.2
CMW0	0.67	29.7	27.3	19.3	16.0	7.00	57.0	76.3
CPMW	1.26	21.6	29.4	23.1	19.1	5.53	51.0	74.1

Table 5 compares the volume ratios between three main phase-constituents. In the case of two forms of calcium-silicate-hydrate, however, the volume ratios are very low compared with those reported by other authors (Kostandines *et al.*, 2003; Kostandines *et al.*, 2004; Mondal *et al.*, 2010; Nemecek, 2012; Hu and Li, 2015). Nevertheless, their changes help in detecting the impact of heat and pressure in the phase-composition of the sample.

**Table 5.** Volume ratios between the main phase-constituents of the samples, with respect to elasticity modulus

Sample	CSH LD/CSH HD	CSH-s/CH
C000	0.034	0.714
CMW0	1.085	2.948
CPMW	0.735	2.207

#### 4. DISCUSSION OF THE RESULTS

Table 4 shows that the non-treated sample (C000) does not contain any nanoporosity, whereas it is present at the MW-cured sample (CMW0). The percentage of nanoporosity increases from the MW-cured sample (CMW0) to the one which is MW-cured and pressed (CPMW). Also the pressing of the sample increases its nanoporosity. It is worth to recall here that the nanoporosity is gel pores ( $\leq 2\text{nm}$ ) that are thought to be intrinsic feature of the C-S-H phase (Diamond, 1985). The trend of nanoporosity increment from C000 to CMW0 and CPMW is in line with the increment of the amount of low density C-S-H gel (table 4). It seems that the nanoporosity which is present at the MW-cured sample belongs to the low density C-S-H gel formed by the pozzolanic reaction and that of the MW-cured and pressed sample comes mostly from the low density C-S-H gel and additionally from the densification of the overall structure (phase formation in a more confined space). The lower density C-S-H gel (outer product) is formed near the unreacted cement during the later stage (Jennings, 2000). Whereas the high density C-S-H is formed from the hydration reaction and its percentage is higher at the MW-cured samples. The above is consistent with the fact that the MW-curing increases significantly the percentage of total C-S-H at early age and specifically of the lower density C-S-H. The pozzolanic reaction proceeds at the expense of portlandite, leaving almost intact the anhydrous

phase (table 4). There is about 28.7% more C-S-H low density phase at the MW-cured sample (CMW0) which is comparable with 23.4% portlandite phase that is consumed by the pozzolanic reaction. With the CPMW sample the corresponding figures are respectively about 20.6% and about 19.6% as compared to the non-cured sample (C000). There is about 46.5% more total C-S-H phase content at the MW-cured sample and about 40.2% more C-S-H phase at the MW-cured and pressed sample compared to the non-treated sample (C000). The total increase of C-S-H phase content (almost double) at the microwave cured sample seem to be the main reason for about 150% compressive strength increase in comparison with the non-cured specimen (Korpa and Trettin, 2008). The lower total C-S-H volume ratio of the CPMW sample (table 5) that is MW-cured in presence of pressure may be due to the shift of the equilibrium according to Le Châtelier's principle, given that pozzolanic reaction is accompanied by increased volume of the reaction products. There are other reasons as well for the very high early strength of the microwave cured samples apart from the higher total C-S-H phase content.

## 5. CONCLUSIONS

The statistical nanoindentation assessment of volume fractions of main phase-constituents of ultra-high performance concrete containing nanoscale pozzolan and cured with microwave energy has revealed an increase of the total C-S-H phase content of the microwave cured samples. There is about 46.5% more total C-S-H phase content at the MW-cured sample and about 40.2% more C-S-H phase at the MW-cured and pressed sample compared to the non-treated sample (C000). The curing with microwave energy mainly increases the content of low density C-S-H at the expense of portlandite phase through the well-known pozzolanic reaction. Additional investigations are needed in order to better understand the reasons for such high strength and the relationships nano- and molecular structure of C-S-H with the macroscopic properties of the microwave cured ultra-high performance concrete that contains nanoscale pozzolans.

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## ANGIOGENESIS IN THE CORPUS LUTEUM – SOME MOLECULAR MECHANISMS OF REGULATION

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### ABSTRACT

Angiogenesis (neovascularisation) - the generation of new capillaries through sprouting from already existing blood vessels in adults is under multiple controls of different endocrine and local produced factors. Of the numerous local promoters of angiogenesis that have been identified, the most important appear to be: vascular endothelial growth factors (VEGF and receptors VEGFR1 and VEGFR2), and the angiopoietin system members (ANPT-1, ANPT-2 and receptors Tie1 and Tie2). Bovine corpora lutea (CL) in our experiments were assigned to the following stages: days 1-2, 3-4, 5-7, 8-12 13-16, >18 of the estrous cycle. The expression of mRNA was measured by reverse transcription polymerase chain reaction (RT-PCR), hormone concentrations by ELISA and localization by immunohistochemistry. The highest expression for VEGF-A (VEGF) in CL was found during the early luteal phase (days 1-4) followed by a lower plateau afterwards (during mid-luteal and late-luteal phase and during corpus luteum regression). The ANPT-2/ANPT-1 expression was high found during the early luteal phase (days 1-4) followed by a lower plateau during mid-luteal phase and increased again during CL regression. The results suggest the importance of VEGF and angiopoietins (ANPT) for angiogenesis, maintenance and angiolytic of capillary structures in CL during different developmental and functional stages. In addition our studies in the bovine ovary tissue offer an excellent model for studying and understanding molecular mechanisms of angiogenesis under physiological conditions, especially during corpus luteum formation and function and regression.

**Keywords:** Angiogenesis, angiogenic factors, VEGF, angiopoietins, mRNA expression, corpus luteum, bovine ovary

## 1. INTRODUCTION

Angiogenesis (neovascularisation) is the preferred term for processes leading to the generation of new blood vessels through sprouting from already existing blood vessels in a process involving the migration and proliferation of endothelial cells from pre-existing vessels (Augustin, 1998; Abulafia and Sherer, 2000; Berisha and Schams, 2005). Recent studies demonstrated that angiogenesis is dependent upon the activity of extracellular matrix (ECM) proteases like MMP (matrix metalloproteinases) and PA (plasminogen activators). The angiogenesis includes a series of steps involving (I) the breakdown or degradation of the basement membrane of existing blood vessels; (II) the migration of endothelial cells into the interstitial space towards an angiogenic stimulus; (III) the proliferation of endothelial cells; and (IV) the formation of new capillary lumina and functional maturation (Augustin, 1998; Clapp *et al.*, 2009). Inhibition of any of key regulators during any step of the angiogenic cascade has been effective in experimental animal models. Angiogenesis is a complex process in which a delicate balance between promoters and inhibitors is maintained. Disturbance of this balance may result in a disrupted physiologic state or various pathologic conditions such as tumor growth and metastasis (Augustin, 1998; Fraser and Lunn, 2000).

In the recent years, nanotechnology is gaining on importance for its application in biotechnology and biomedicine. The application of nanotechnology to medicine, known as nanomedicine, concerns the use of precisely engineered materials at this length scale to develop novel therapeutic and diagnostic modalities (Navalakhe and Nandedkar, 2007; Zhang *et al.*, 2008). Indeed, current nanotechnology-based therapeutic products have been validated through the improvement of previously approved drugs, and many novel classes of nanotherapeutics are now underway (Zhang *et al.*, 2008; Shi *et al.*, 2010). Some recent publication summarizes key factors targeted for therapeutic angiogenesis and anti-angiogenesis gene therapy, non-viral nanoparticle-mediated approaches to gene delivery, and recent gene therapy applications in pre-clinical and clinical trials for ischemia, tissue regeneration and cancer (Kim *et al.*, 2016; Dhruba and Shaker, 2013; Langer *et al.*, 2013).

### 1.1. Angiogenesis and ovarian function

Formation of the corpus luteum (CL) is initiated by a series of morphological and biochemical changes in cells of the theca interna and granulosa (luteinization) of the preovulatory follicle (Schams and Berisha, 2002; Berisha *et al.*, 2016). The growth of follicle or new CL tissue depends

upon growth of new blood vessels and establishment of a functional blood supply. In the mature CL nearly every parenchymal cell is in contact with one or more capillaries (Redmer and Reynolds, 1996).

Angiogenesis in the reproductive tract, and especially in the ovary, is nowadays well established to be of great importance for its development and function (Reynolds and Redmer, 1999; Fraser and Lunn, 2000; Berisha and Schams, 2005). The ovarian cycle by ruminants is characterized by repeated patterns of specific cellular proliferation, differentiation and transformation that accompanies follicular development and the formation and function of the corpus luteum. In addition, because the CL is so dynamic, it provides an ideal model for studying the regulation of the very complex process of angiogenesis, as the most important regulatory event for follicle and corpus luteum development and function (Reynolds and Redmer, 1999; Fraser and Lunn, 2000; Berisha and Schams, 2005). An improved understanding of the role of angiogenesis during follicular growth and CL formation and function obviously has important implications for the regulation of fertility in mammals (Fraser and Lunn, 2000; Prokopiou *et al.*, 2013; Berisha *et al.*, 2016).

### **1.2. Angiogenic Factors in the Ovary**

The ovary was among the first organs in which local produced growth factors with angiogenic activity were detected (Gospodarowicz and Thakral, 1978). Of the numerous promoters of angiogenesis that have been identified, the most important factors appear to be vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), angiopoietins (ANPT), hypoxia-inducible factor (HIF) family members (Abulafia and Sherer, 2000; Hanahan, 2007; Clapp *et al.*, 2009; Berisha *et al.*, 2016).

### **1.3. The objective of the study**

The objective this paper is to discuss some mechanisms of regulation, which are important for angiogenesis during corpus luteum formation and function in the bovine ovary. The different angiogenic family members were shown to regulate angiogenesis, but expression of VEGF and angiopoietin family members seems to be the most important molecular mechanisms of vascular regulation: angiogenesis, vascular maintenance and angiolytic (Reynolds and Redmer, 1999; Fraser and Lunn, 2000; Berisha and Schams, 2005). An improved understanding of luteal regulation by VEGF and angiopoietin system could yield novel strategies for manipulating the ovarian vasculature to improve fertility performances in this species.

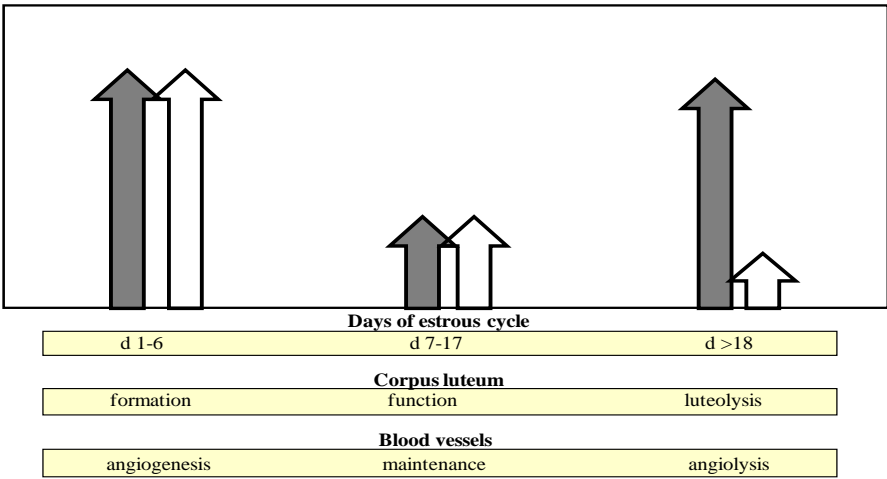
## 2. MATERIAL AND METHODS

Entire reproductive tracts from cows were collected at a local slaughterhouse. The stage of the estrous cycle was defined by macroscopic observation of the ovaries (follicles and corpus luteum). The CL was accordingly assigned to the following stages: days 1-2, 3-4, 5-7, 8-12, 13-18 and >18 (after regression) of estrous cycle. The expression of mRNA for the ligands and receptors were evaluated by means of rRT-PCR and ribonuclease protection assay (RPA), hormone concentrations by RIA and localization by immunohistochemistry (Berisha *et al.*, 2000).

## 3. RESULTA AND DISCUSSION

### 3.1. Vascular endothelial growth factors (VEGF) in the bovine ovary

Vascular endothelial growth factor family members (VEGF-A, VEGF-B, VEGF-C and VEGF-D) bind their specific VEGF receptors (VEGFR1, VEGFR2). It is now well established that alternative exon splicing of a single VEGF-A (VEGF) gene results in the generation of different molecular species, having respectively 121, 165, 189 and 206 amino acids after signal sequence cleavage (Berisha *et al.*, 2016). Bovine VEGF is one amino acid shorter than the human factor. VEGF expression is influenced by numerous factors including hypoxia, hormones, growth factors and cytokines. The biological activities of VEGF are mediated through high affinity receptor tyrosine kinases (VEGFR1, 2 and 3) genes, largely restricted to the vascular endothelium. VEGF has been shown to regulate most steps of the angiogenic process, including extracellular matrix degradation, endothelial cell migration, proliferation, and tube formation (Redmer *et al.*, 1996). The mRNA expression data for VEGF and its receptors in bovine CL during estrous cycle are schematically shown in Fig. 1.



**Fig.1.** Schematic presentation of expression profiles of ANPT-2/ANPT-1 ratio (black bars) and angiogenic factor VEGF (white bars) during angiogenesis, maintenance and angiolysis of blood vessels in bovine corpus luteum during different functional stages (Modified according to Berisha and Schams, 2005).

The highest VEGF mRNA expression in CL was detected during the period of angiogenesis (early luteal phase). The expression intensity of VEGF decreased significantly after Day 7 to a lower plateau and further after regression of CL. The VEGF protein concentration in CL extracts by RIA showed a similar trend as for the mRNA expression (Berisha *et al.*, 2000). Comparable results were obtained in sheep CL (Redmer *et al.*, 1996). A strong staining for VEGF is seen in macrophages of the corpus luteum. Also the endothelium and the smooth muscle cells of blood vessels are distinctly immunostained (Berisha *et al.*, 2016).

### 3.2. Angiopoietin (ANPT) family members in the bovine ovary

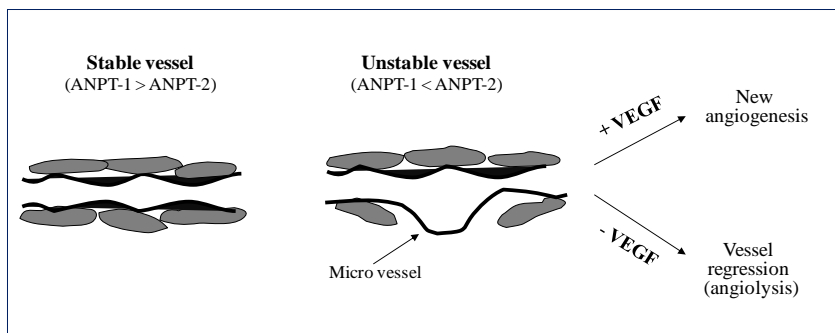
The components of the angiopoietin system (ANPT-1, ANPT-2 and their receptors Tie1 and Tie2) play important roles during CL development and function. The RT-PCR analyses in bovine CL showed that both ANPT-1 and ANPT-2 and their receptors were expressed during estrous cycle. Our data support the earlier results of Goede *et al.*, (1998). In early and regressing CL, the ANPT-2/ANPT-1 ratio was higher than those of mid CL (Fig. 1.). This resulted in a decrease in the ANPT-2/ANPT-1 ratio, indicating that the blood vessels became more stable or mature. The increase of the ANPT-2/ANPT-1 ratio during early luteal stage in our experiments seems to be a basic mechanism of vascular remodeling in after ovulation and CL formation (Tanaka *et al.*, 2004; Hayashi *et al.*, 2004; Shimizu *et al.*, 2007; Berisha *et al.*,

2016). The results of angiopoietin expression in CL during estrous cycle are schematically presented and summarized in Fig. 1, and suggest an important role of these growth factor systems in vascular formation (angiogenesis) and regression (angiolysis) during CL function. A recent finding suggests that ANPT-1, ANPT-2 and their receptor kinases Tie1 and Tie2 may play an important role for the modulation of angiogenesis and angiolysis in the CL during the estrous cycle (Hanahan, 1997; Goede *et al.*, 1998; Berisha *et al.*, 2016).

#### 4. CONCLUSIONS

The schematically presented data for VEGF and ANPT family members in bovine CL are summarized in Fig. 1. The data suggests an important role of these systems during CL formation and function. Furthermore additional maintenance functions of these factors for capillary endothelial cells have been postulated. VEGF is required to inhibit apoptosis of the endothelial cells and is essential for the stabilization of the newly formed blood vessels (Alan *et al.*, 1995). Generally, the ANPT-Tie system is thought to act in concert with growth or survival factors such as VEGF. The ANPT-1 is necessary to maintain and stabilize blood vessels (Yancopoulos *et al.*, 2000). On the contrary, ANPT-2 which acts as natural antagonist for ANPT-1, appears to cause endothelial cell (EC) to undergo active remodelling, thus it destabilizes vascular structure. As ANPT-1 and ANPT-2 bind to the same receptor Tie2, the ratio of ANPT-2/ANPT-1 appears to play a crucial role for vascular stability. The high ANPT-2/ANPT-1 ratio in microenvironment induces destabilization of blood vessels, which is a prerequisite for vascular formation and angiolysis (blood vessel regression). In such conditions, the presence of angiogenic factor such as VEGF could determine the fate of destabilized blood vessel (Fig. 2).

When VEGF is high, a destabilization of blood vessels results in the formation of a new vascular network, whereas a lack of VEGF results in a regression of blood vessels (Hanahan, 1997; Yancopoulos *et al.*, 2000; Berisha *et al.*, 2016).



**Fig. 2.** Schematic presentation of regulation mechanisms of vascular function. The concept for angiopoietins (ANPT) and VEGF in vascular modulation (Angiogenesis, maintenance and angiolytic) during capillary function in bovine corpus luteum (Modified according to Hanahan 1997).

In conclusion our results suggest the importance of VEGF and angiopoietins (ANPT) for angiogenesis, maintenance and angiolytic of capillary structures in CL during different developmental and functional stages. In addition our studies in the bovine ovary tissue offer an excellent model for studying and understanding molecular mechanisms of angiogenesis under physiological conditions, especially during corpus luteum formation and function.

Recent publications focus on applications of nanoparticles for gene therapy to modulate angiogenesis (Zhang *et al.*, 2008; Langer *et al.*, 2013; Kim *et al.*, 2016). Dhruva and Shaker (2013) gave a description of some of the nanotechnology-based therapeutic angiogenesis strategies used in recent years. Taken together, the studies emphasize the potential use of non-viral gene delivery nanoparticles for the modulation of angiogenic processes. Therefore, there is a need to further develop our understanding of non-viral gene nanoparticles and the biology of angiogenesis to optimize the selection and delivery of genetic material to target tissues for the treatment of angiogenesis-dependent diseases (Zhang *et al.*, 2008; Dhruva and Shaker, 2013; Langer *et al.*, 2013; Kim *et al.*, 2016).

## ACKNOWLEDGMENTS

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## BIOSENSORS AND TECHNOLOGIES USED IN MONITORING PATIENTS

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### ABSTRACT

Nowadays the process of taking care of patients is focused in increasing accuracy of monitoring the disease. Sensors are very useful in monitoring process because they can detect patient health status in real time. Sensors are used in chronic diseases, in hospitalized and out-patients. There exist different sensors situated within the body that can be used to monitor patients' health according to their diseases. However the advances in nanotechnology makes possible to monitor luminal organs such as blood vessels, ventricles of the brain, etc. This offers the opportunity to evaluate processes of different diseases and monitor chronic illness. In this paper, will be discussed the technologies used for monitoring patients health through sensors.

**Keywords:** biosensors, health, technologies

### 1. INTRODUCTION

Sensors play an important role in monitoring patients and extract information about their health status. Biosensors are chemical sensors in which the recognition system functions via a biochemical mechanism (Boehm, 2014). They can provide information on general health of patients. Sensors are used in generating data in real time. Biosensors employ biological molecules to recognize the target and utilize output elements. The term 'biosensor' is often used to cover sensor devices which are used to determine the concentration of substances and other parameters of biological interest (Atta *et al.*, 2011). The output elements utilized by biosensors can translate the biorecognition event into electrical, optical or mass-sensitive signals to determine the quantities of the target (Chao, *et al.*, 2015). This paper presents the advantages of using sensor technologies and gives examples of sensors and their use in monitoring health. Sensors can be used for monitoring blood glucose level, blood pressure, pulse rate, respiration rate (Yilmaz *et al.*, 2010) as well as to detect patients with Parkinson's disease (Perumal and Sankar, 2016). Sensors provide a good way in monitoring patients in a remote way and assist in managing chronic diseases.

## 2. Use of biosensors in health monitoring

Biosensor are used most in health monitoring because they can provide real time, very accurate evaluation of parameters. They can be used by the doctors to obtain the correct results. The role of biosensors in healthcare is to collect data from different processes and then to transmit the data to a device that will monitor the patient. Biosensors can be categorized into four different diagnostic types, depending on their use or physical location: (Arnold *et al.*, 2014).

**Table 1: Categorization of Biosensors**

Diagnostic type	Use	Analyze
Non – invasive	electromagnetic radiation to "see inside" or the use of smart phones	motion and activity
In vitro	Laboratory	fluids outside of the body such as sweat or saliva
Transcutaneous	Placed on the skin	heart rhythm
Ingested	Example pill swallowed	internal body temperature or to visualize the colonic mucosa
Wearables	Clothing and worn such as hat, shirt, band, watch, glasses, or a pair of shoes	

Biosensors are very accurate. They can monitor chemical or physical changes that occur in the patients' body. Taking data from the patients will give the opportunity to the doctors to diagnose and to treat different illness of the patients. There are a lot of advantages if the patients monitor and detect illness at the first stage because they can reduce the cost and can prevent it. From a diagnostics view point, accuracy of the diagnosis is vital in terms of the kind of therapeutic to be used. The major concern in diagnosis is patient compliance where invasive samples (blood and tissue) are routinely taken to analyze the severity of the illness. (Ngoepe *et al.*, 2013)

There are a lot of sensors that are used to measure the health of the patients. Some of them are electrocardiogram sensor (heart activity), an electromyogram sensor (muscle activity), electroencephalogram sensor (brain electrical activity), breathing sensor (respiration), a blood pressure sensor (blood pressure), a tilt sensor (trunk position), motion sensors (level of activity).

Diabetes is a disease where the blood concentration of glucose is too high. The glucose biosensor is used to monitor the blood glucose. It is crucial to maintain the glucose blood level within normal range in order to prevent further multiorgan complications of this disease.

Cholesterol is a crucial parameter that can be used in the diagnosis of coronary heart disease or arteriosclerosis. The clinical analysis of cholesterol in serum samples is important in the diagnosis and prevention of a large number of clinical disorders such as hypertension, cerebral thrombosis and heart attack. There are studies on utilization of biosensors for detecting cholesterol level. (Ahmadalinezhad and Chen 2011). The tested biosensors showed a wide range up to 30mg dL<sup>-1</sup> in a normal human body condition (pH 7.4). This is very promising for the determination of cholesterol in clinical assets.

To date Parkinson's disease is diagnosed by clinical assessment from specialized doctors, namely neurologists. New research has discovered the possibility of biosensors to early detect levels of dopamine (the substance produced by the brain stem, which is depleted in Parkinsonian patients. This will permit a better and faster diagnosis and treatment of this debilitating disease in early stages.

The table 2 describes eight locations where the patients can locate the sensors, what information can be collected from the sensors and what type of sensor is used to collect that information.

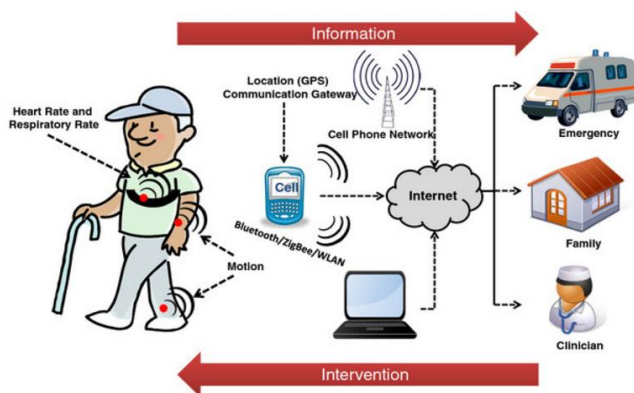
**Table 2:** Examples of sensors

No.	Location	Type of Sensor	Used to measure
1	Wrist	Ultrasound	Blood pressure
2	Finger	Radio-frequency identification (pulse & temp.) (Mathie, Coster, Lovell, & Celler, 2004)	Heart rate and temperature
3	Phone adapter	Single-channel ECG37 (Paul, David, & Dwight Reynolds, 2012)	Heart rate and rhythms
4	Arm	Multi-variable (Zanon, et al., 2012)	Blood glucose
5	Eye	Glucose (Forbes, 2014)	Ocular glucose
6	Clothes	Inertial sensors and accelerometers (Rand et al.2009) (Kuo, et al.2009)	Walking distance, stride distance, and step count during stair ascent and descent
7	Wrist or ankle	Accelerometer and motion (Lockman, Fisher, & Olson, 2011)	Seizure activity
8	Ankle	Pedometers and accelerometer	Walking distance and step counts

### 3. Technologies used in wearable systems for patients

There are many wearable systems used to monitor patients. From these systems can benefit different actors. Clinical staff can monitor remote patients

in real time and make decisions about their treatment. The family of the patients can get information and alert in case of emergency. Also the emergency can get an alarm message about emergency situations of the patients in order to assist the patients as soon as it is possible. A conceptual representation of a system for remote monitoring is shown in Figure 1 (Patel *et al.*, 2012).



**Fig. 1:** Health Monitoring System Illustration (Patel, et al., 2012)

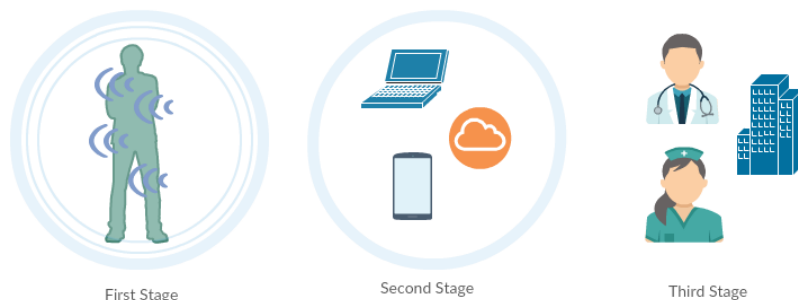
In this system the patient is having wearable sensors. These sensors are used to monitor the status of the patients. There are used different sensors according to the information that it is going to collect from the patient. For example to monitor heart failure or obstructive pulmonary disease are deployed sensors to monitor heart rate, respiratory rate etc. Sensors for detecting movement can also be used to monitor home-based rehabilitation interventions in stroke survivors or the use of mobility assistive devices in older adults. To transmit the information of the patients to a mobile phone or an access point it is used Wireless communication over internet.

The system of monitoring patients in wearable systems according to Patel, et al., (2012) consist of 1) the sensing and data collection hardware to collect physiological and movement data, 2) the communication hardware and software to relay data to a remote center, and 3) the data analysis techniques to extract clinically-relevant information from physiological and movement data.

WBAN (Wireless Body Area Network) are sensors that are placed in the patients so the doctors can gather data about the patient. WBAN sensors are located on the body or inside the body to measure different parameters. They offer the possibility to monitor the patient in real time and take health decisions. Because the data gathered from patients are increasing every day

and to make a system where the data are secured it is better to integrate WBAN, IoT and cloud computing. Internet of Things (IoT) has the technology to collect and exchange data. It can create an intelligent healthcare system and patients will be monitored more effectively. Cloud Computing provides a lot of services with high storage capacity. It can be used to store, manipulate and analyze data taken from sensors. The data on the cloud can be accessed everywhere and from physicians, doctors, patients etc. to monitor in real time the health of the patient.

WWBANs (Wearable Wireless Body Area Network) are very important in health care. The architecture has three stages as shown in figure 2: wireless nodes of medical sensors, personal server applications and medical server.



**Fig. 2:** Typical WWBAN architecture

The first stage includes medical sensors. The nodes process physiological signals. These sensors will be used for monitoring blood pressure and heart, brain, muscle, respiration and motion activity.

The second stage includes the personal server application on mobile phone, laptop or pc. It offers the user interface for wireless sensors, medical server and users. It includes configuration, management, registration and security settings.

The third stage includes a medical server access via the Internet. In addition to the medical server, the last tier may include other servers, such as informal caregivers, commercial providers of healthcare and even emergency servers. The medical server typically runs a service that sets up a communication channel for the personal server to users, collects reports from user, and integrates the data in the medical record of the user. (Darwish & Aboul, 2011)

### 3.1 Nanotechnology in health diagnosis

Nanotechnology is used to be able to detect early signs of diseases. There are optical nanosensor, based on single walled carbon nanotubes that

modulate their emission in response to the adsorption of specific biomolecules, can be used as a glucose sensor (Mano and Heller, 2005). Nanotechnology can also be used for diagnosing infectious diseases. Magnetic nanobeads as a nanosensor for clinically relevant viruses and bacteria can be used to can be used for diagnosing infectious agents. Infectious agents might cause SARS, dengue etc. (Khalid and Yazeed, 2014). Nanotechnology play an important role also in diagnosis tuberculosis. The diagnostic principle of nanomechanical deflection of the microcantilever due to adsorption of the TB specific antigens on its upper surface is employed for the diagnosis of TB. The deflection of the microcantilever would be measured in terms of piezoresistive changes by implanting boron at the anchor point (Nelson *et al.*, 2001). Nano- and microparticle-based imaging of cardiovascular interventions is still in its developing phase. It has already presented exciting potential to monitor primary interventional procedures for precise therapeutic delivery, enhance the effectiveness of delivered therapeutics, and monitor therapeutic efficiency after interventions performed to treat cardiovascular diseases (Xiaoming, 2007).

Nanodevices and especially nanowires can detect cancer-related molecules, contributing to the early diagnosis of tumour. Nanowires having the unique properties of selectivity and specificity, can be designed to sense molecular markers of malignant cells. They are laid down across a microfluidic channel and they allow cells or particles to flow through it. Tumour treatment can be succeeded with nanoscale devices (such as dendrimers, silica-coated micelles, ceramic nanoparticles, liposomes). These devices can serve as targeted drug-delivery vehicles capable of carrying chemotherapeutic agents or therapeutic genes into malignant cell (Abber, 2012).

#### 4. CONCLUSIONS

Using sensors in health-care system can reduce the cost, can prevent diseases and can improve the quality of life. Sensors play an important role in managing diseases. An early an accurate measurement and monitoring of diseases will be very useful for their treatment and control. Biosensors provide positive impact in diagnosing, monitoring and maintaining health. Furthermore the automated, passive or active collection of data and first level automated analysis of that data through the described systems can aid in the management of chronic and episodic conditions such as Diabetes, Congestive Heart Failure, Parkinson etc. (Arnold *et al.*, 2014). The enabling technologies developed over the past decade have facilitated the deployment of wearable sensors and systems for clinical applications. WBAN are integrated with IoT



and cloud to store, process the data to produce an efficient telemedicine system. Their integration can be very useful in remote monitoring of patients.

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## FLAT AND STEPPED SURFACES MIMICKING NANOPARTICLES

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### ABSTRACT

Nanoparticles are used effectively in reactions as catalysts, biosensors, chemical sensors. In order to get a better understanding of the synthesis and interaction of nanoparticles researchers have combined computer simulation with atomic-resolution imaging techniques. The limitation of the experimental techniques at the nanoscale and the limitation of the computational techniques to cover all the relevant interactions has led the efforts to the reduction of the complexity of the systems and the use of approximate methods. Once the experiment and the simulation meet, then one can theoretically scan a whole range of nanomaterials and predict the best one for a specific reaction. Theorists using the computationally demanding density functional theory (DFT) can predict the best active sites but it is inherently very difficult to quantify these sites on largely inhomogeneous nanoparticles. One approach is to model the interaction of small molecules with nanostructured macrostructures. Reactions on metal and semiconductor surfaces provide an important benchmark for the study of heterogeneous catalysis and theoretical calculations. The current experimental capabilities have enabled a close to ideal control of the degrees of freedom for both the surface and the molecules in its proximity. Recently, experimentalists have worked on carefully prepared stepped surfaces with terraces of few atoms wide, which provide spatial control in the order of few nm. The stepped surfaces have nanosized patterns that mimic the geometry of a nanoparticle. This possibility may identify important step types and step geometries that exist on nanoparticles and that are ideal for heterogeneous catalysis or other important application. In this review we present some of the experimental techniques that are employed in ultrahigh vacuum (UHV) to analyze the interaction of molecules with stepped surfaces.

**Keywords:** Catalysis, Stepped surfaces, Nanoparticles, Nanostructured surfaces, Reactivity.

## 1. INTRODUCTION

The aim of this work is to provide a general treatment of the surfaces and how they can be used to get a better understanding of the nanoparticles. Their physical and chemical properties of nanoparticles are size dependent because of: i) the high number of surface atoms when compared to the number of bulk atoms, and ii) quantum confinement. The melting point of a metal nanoparticle is different from the melting point of a large structure because of the first reason. When the particle size is comparable with the exciton radius that is associated with an excited electron then electronic structure becomes size dependent because of quantum confinement. For silicon the exciton radius is 5 nm (Kolasinski, 2012) and at this scale any reaction that involves the sharing or transfer of the electron will depend strongly on the size. Most of the reactions occur on nanoparticles that have defects of different orders and have areas with different coordination number. When a metal is used in catalysis for specific processes, the reactions may be completed on perfectly flat regions that are with a high density or that cover approximately 98-99% of the total area, or on defects that are present with a much smaller density down to even 1% or less. The reaction of interest may be the breakdown of a specific diatomic or larger molecule, or the formation of a larger from smaller constituents. The reactivity of a perfectly flat surface may be in the order of  $10^{-4} - 10^{-3}\%$  which is defined as the ratio of the molecules that undergo the reaction of interest to the total number of molecules. In this case we assume that we can have a good control of the flux of the molecules over a surface of interest. As it will be explained in more detail further in this review, such control is possible in ultra-high vacuum (UHV) machines. As a rule of thumb, the flux of the incoming molecules ( $\phi \left( \frac{\text{molecules}}{\text{cm}^2} \right)$ ) should be smaller than the density of the sites on a sample. The density of the sites on a surface is in the order of  $10^{15} \left( \frac{\text{sites}}{\text{cm}^2} \right)$ . The good control of the flux of the molecules on the surface that plays the role of a catalyst and the control of the rate of reactions allows the researchers to measure very small reactivity rates, or at least to put an upper boundary in case they are too small. The reactivity on a defect site may be in the order of 10 – 50%. If we do not conduct a careful analysis of the observed process or of the reaction that we are interested in, it may be attributed to the whole surface of the metal. The high reactivity (or reaction probability) on the low-density defect sites may often mislead one to assume that the flat surface was responsible for the observed overall reaction. On catalysis often the nanoparticles are used and they are full of defect sites or step sites. Researchers in the effort to have complete understanding in the site-specific reaction rates and to design the future catalyst surfaces have long tried to understand the reactions and model them. In order to model surfaces,

the theorists need to have as less complexity as possible and for this reason they require from the experimentalists measurements from systems that are as simple as possible. As the complexity of the surface-molecule is reduced, the researchers are then able to come closer to a complete atomic-scale understanding of the reaction and this would facilitate the modeling of the next generation of catalysts. Theorists have been able to determine rates of complex reactions on catalytic surfaces starting from atomic scale calculations (Honkala et al, 2005; Hellman et al., 2006; Howes et al., 2014; Makarucha et al., 2011). Kolasinski boldly phrased for the first time the idea that surface science is the area where heterogeneous catalysis and nanotechnology meets (Kolasinski, 2012). To imitate the defects on a surface or regions with a specific coordinative number that could be present on nanoparticles, one can use flat surfaces that are cut at different angles and produce stepped surfaces. The preparation of stepped surfaces with the required orientation in large sizes is now affordable thus enabling reasonable experimental conditions. A further positive development is the use of cylindrically shaped single crystals that makes possible to have several surfaces with different orientations all at once. The use of molecular beams and the control over the degrees of freedom allows quantum state-selective measurement of reactivity and scattering parameters.

In the following sections we will firstly discuss the flat, the reconstructed and the stepped surfaces (that have both terraces and steps). In the next section we will discuss the methods of analyzing of the surfaces. Then we will follow with the experiments that provide concrete results that contribute in the determining of the site-specific reactivity.

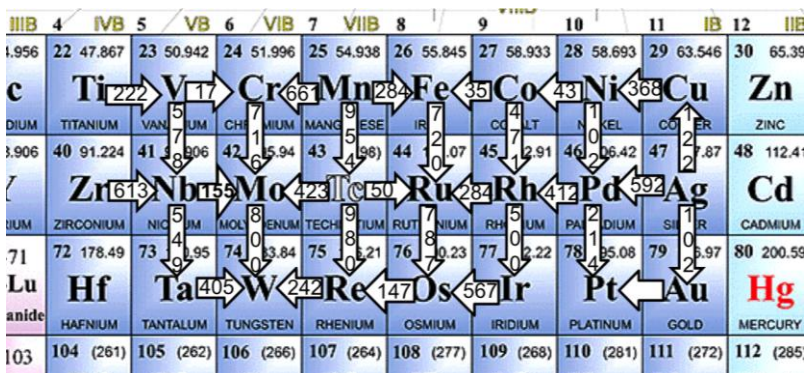
## 2. EXPERIMENTAL

The data that we show for flat copper and stepped platinum are taken on two different experimental setups. Both samples are approximately of the same size: around 1 cm in diameter. Samples are heated by electron bombardment emitted from a tungsten filament and are cooled conductively using liquid nitrogen that flows through the sample holder. The sample holder is made of copper. The cleaning cycle for copper includes argon ion sputtering for 30 minutes while the sample is kept at 550°C, and is followed by annealing for 30 minutes while the is kept at 600°C. After the cleaning procedure, Auger electron spectroscopy (AES) and low energy electron diffraction (LEED) confirmed a clean surface with no contaminants and sharp LEED pattern. Copper has a high barrier to hydrogen dissociation and in order to study the hydrogen covered copper we exposed the sample to a hydrogen flow that was introduced into the UHV chamber through a clean molybdenum tube. The hydrogen molecules as they leave the tube, flow over a heated

tungsten filament that is kept at temperatures of above 2000°C and this ensures that the sample is covered by hydrogen, a fact confirmed by temperature programmed desorption. The cleaning of the stepped platinum surface was done by three repeated cycles of argon ion sputtering (15 A, 5 min) and annealing in partial pressure of oxygen ( $2 \times 10^{-8}$  mbar) while the sample is kept at 850K. After the three cycles of sputter-annealing the sample was annealed for 3 minutes at 1200K. This cleaning procedure produced sharp LEED patterns with distinct double spot that has to do with the characteristic of the (553) plane. In order to do TPD measurements, hydrogen was dosed by background dosing while the sample is kept at 150K. The heating rate was  $2Ksec^{-1}$ . The background pressure in the main chamber was  $5 \times 10^{-10}$  Torr.

### 3. FLAT SURFACES

Heterogeneous reactions happen when two different phases meet such as the crystal and gas. Researchers are mostly interested in transition metals as they have served as important catalysts in reactions such as ammonia synthesis and Fischer-Tropsch reaction. Eventhough the majority of the transition metals have a face-centered cubic (FCC) crystallographic structure and they deservedly have a lot of attention, the two important reactions mentioned above prefer iron that has a body centered cubic (BCC) structure as a catalyst. The interatomic forces and many other chemical properties that appertain more to the bulk change as a function of the atomic number. For example, the melting point of all the metals is shown in Fig. 1. Silver (Ag) has the lowest melting point (960°C) among all the metals whereas tungsten (W) has the highest melting point (3420°C). Carbon that is a nonmetal has the highest melting point among all the materials. We observe that between any two subsequent periods, the melting point increases by  $10^2 - 10^3$ °C in the latter while in the same group, with the sole exception being the jump from silver to copper. When the groups in the periodic table are compared we observe that group VI and group VIII have the highest melting points. This is the general case for pure metals. Compounds that contain metals and carbon have been observed to have higher melting points (Hong, 2015). Just like the bulk structure affects many properties of the matter, the surface structure too affects other ones. One of the most important and essential processes that happen on the surface is adsorption. Adsorption is a process that depends on the structure of the surface and experiments until now have shown that as a process it may change from one group to another. For example, on the elements of group I-B (copper and gold) the adsorption of hydrogen is strongly activated, whereas on the elements of group X (palladium and platinum) the adsorption has much lower energetic barriers.



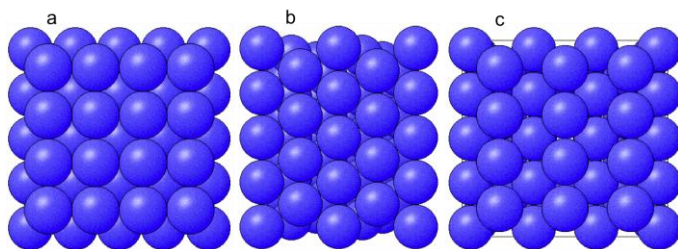
**Fig. 1:** The sketch of the differences of the melting point from one element to the other

The surface is defined as the top few layers of the metal and in general the atoms on the surface follow the same periodicity as the bulk atoms. There are cases when the atoms on the surface reorganize in order to minimize the energy and the interatomic distances between the surface atoms become different from those in the bulk. In this situation a reconstruction of the clean surface (a surface with no adsorbates) is observed. Adsorbates can also induce reconstruction on the surface of metals (Chorkendorff, 1991; List et al., 1984).

In Fig. 2 the normal planes of an FCC metal are shown. The (111) plane is the most closely packed one. The (110) plane looks like a (111) plane where every other row of surface atoms is stripped off. The relative surface atom densities for the (100), (110) and (111) planes are 0.86, 0.61 and 1.00, respectively. The approximate atom density is  $1.5 \times 10^{15} \text{ cm}^{-2}$ .

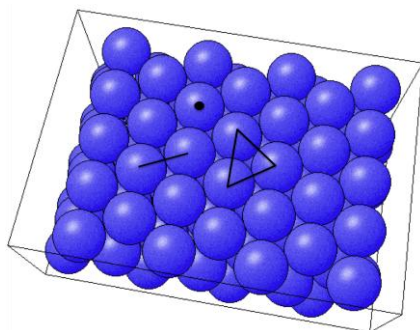
An important concept in surfaces is the corrugation, which is used for both the geometric and the electronic structure. Among the three planes shown in Fig.2 the (100) and (111) seem to be flat surfaces, whereas the (110) plane seems to have a distinct corrugation. The (110) plane could be easily be interpreted as a stepped surface, but it is not exactly the case as it has well-defined sites for adsorption of molecules. The geometric structure of each of the surface planes can be determined by the low energy electron diffraction (LEED).





**Fig. 2** The (100), (111) and (110) planes of FCC metals

Adsorption of atoms or of molecules is a fundamental process on surfaces and it happens on different sites as shown in Fig. 3. A top site is one where the atom (adsorbate) lands right on top of a surface atom. A bridge site is a site halfway between two surface atoms (shown by a line in figure). A threefold hollow site (shown by a triangle in Fig. 3) is one where the adsorbate has a coordination number of 3. The adsorbates create regular structures (distributed with specific periodicity on the surface) on the surfaces and they can be confirmed by LEED (Niu, 1996). When hydrogen is adsorbed on the surface it cannot be observed by LEED as it is not a good electron scatterer. The adsorbates share electrons with the surface atoms and in the process of minimizing the surface energy. An elegant and straightforward way to analyze the binding energies of the adsorbates on the surface is temperature programmed desorption (TPD). In order to perform TPD of a molecule from a surface, one has to first expose the surface to the molecules. The unit of exposure is Langmuir (L) and it is equal to pressure times time, and more precisely  $1L = 1 \times 10^{-6} \text{ Torr} \times 1 \text{ sec}$ .



**Fig. 3** (111) Surface orientation of a FCC metal and the sites for adsorption:  
i) top, ii) bridge and iii) three fold hollow site



The flux of the molecules as a function of pressure is given by  $F_{gas} = \frac{P}{\sqrt{2\pi m_{gas} kT}}$ . Given this relation of the flux with the mass of the gas involved, one can calculate that the time needed to expose a surface to 1 Langmuir of hydrogen, deuterium and oxygen is 0.7, 1.0 and 2.8 seconds, respectively (when the pressure is  $1 \times 10^{-6} Torr$ ). If the reaction probability, or the sticking probability, is unity (all the incoming molecules adsorb on the surface) then the coverage would be 1 monolayer (ML). 1 ML is approximately  $1.5 \times 10^{15}$  adsorbed atoms per  $cm^2$ . One Langmuir (used for the gas exposure) is numerically equal to one monolayer (used for the coverage on the surface). The exposure is done at temperatures lower than the desorption temperature of the gas used. Then the sample is brought close to a mass spectrometer that measures a pressure rise as the sample temperature is increased.

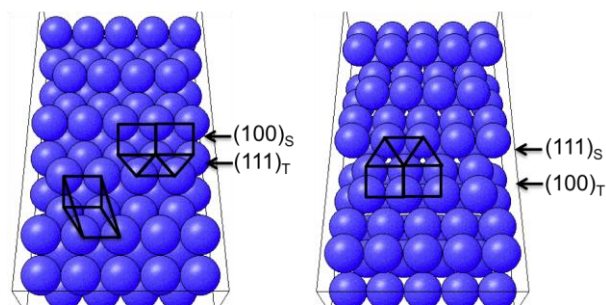
Until now we have discussed only low Miller index surfaces. With the improvement of experimental facilities and with the interest need to have an understanding of nanostructured surfaces the use of stepped surfaces has increased. Stepped surfaces are high Miller index surfaces that can be decomposed into low index terraces and steps. The terraces can be as short as 2 atom. The interatomic distance is approximately 3 angstrom and a control of a terrace with a length of few atom long means that we can obtain experimental results for structures that are around 1 – 10 nm. In the LEED pattern of stepped surfaces, in contrast from flat surfaces, we observe new features that are called dublets that are indicators of the stepped surfaces. Also from TPD researchers have obtained chemical bond information that is specific to a single type of step, or a certain type of step.

#### 4. ANALYSIS OF STEPPED SURFACES

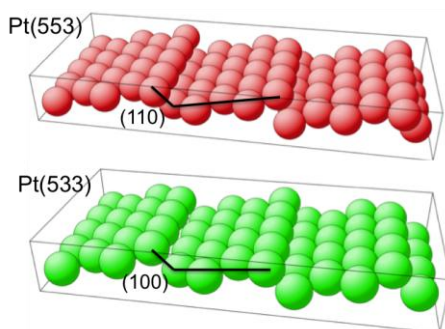
The analysis of low Miller index surfaces is found in all classical textbooks and we will not cover it here. A stepped surface is one that has terraces with low Miller index and steps that can have monoatomic or few atoms height. The major contribution was given by Nicholas and the group of Somorjai (Nicholas, 1965; Lang, 1972; van Hove, 1980). There are two widely accepted notations: i) the step notation and ii) the microfacet notation. In the current literature the step notation is more commonly used as the researchers are interested in the length of the terrace. The general form is  $n(h_t k_t l_t) \times (h_s k_s l_s)$ , where  $n$  represents the length of the terrace,  $h_t$  represents the index of the terrace and  $h_s$  represents the index of the step. Surface (211) has a 3 atom long terrace of (111) plane and one monoatomic step of (100) plane. Some surface may have two different representations in

terms of terrace and step type. For example the (311) plane can be written in two different step notations such as:  $(311) = 2(111) \times (100) = 2(100) \times (111)$ . We can see this in Fig. 4, where the steps, terraces and unit cell is shown. As the compare surfaces (553) and (775) we notice that as the Miller indices increase, the size of the unit cell also increase.

The experiments can measure the effect of the terrace and of the step for the stepped surfaces independently. The two most straightforward techniques are LEED and TPD. Employing two different stepped surfaces with the same terrace length and a different step type enables one to determine the importance of the effect of the step type. A different step type means different coordination number, and also a different Platinum (553) that is denoted as  $Pt(s) - [4(111) \times (110)]$  exhibits different desorption dynamics than platinum (533) that is denoted as  $Pt(s) - [4(111) \times (100)]$ . They both have the same terrace type and the difference in the step type is shown to greatly affect the hydrogen desorption (van der Niet et al., 2010; Groot, Kleyn and Juurlink, 2011). Also, in another interesting work, van de Niet et al. showed that  $Pt(533)$  becomes hydrophobic when hydrogenated, whereas hydrogenated  $Pt(553)$  is wet at the same conditions (van der Niet et al., 2011).

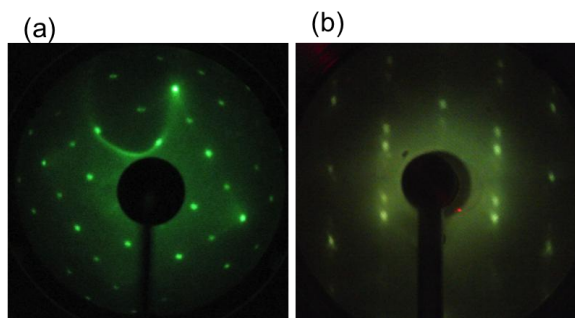


**Fig. 4** Two different step notations are shown for surface (311). On the right the unit cell is also shown.



**Fig. 5** Pt(553) and Pt(533) shown with their step type. Both surfaces have (111) type terrace

In the figure below we show the LEED pattern for a  $Cu(100)$  and  $Pt(533)$  together with the electron beam energy that is used. The pattern for copper is relatively simple as the unit cell is a square and the shape in the reciprocal space will be also a square. The pattern of the stepped surface is dominated by the hexagonal structure of the (111) terrace. We observe also a doublet that is a result of the periodicity of the steps on the surface.

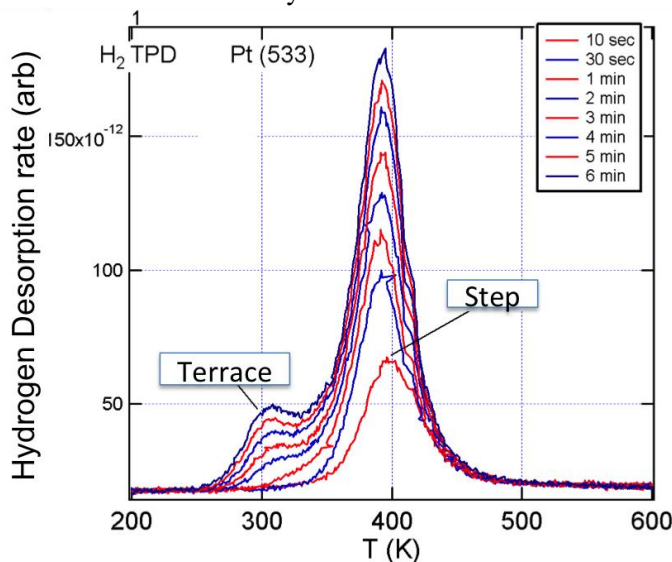


**Fig. 6** LEED pattern of flat and stepped surface:  
(a)  $Cu(100)$ -  $E=178$  eV, (b)  $Pt(533)$ -  $E=350$  eV

The reactivity of the molecules impinging on surfaces depends on the incident angle. In the case of the stepped surfaces there are three normal directions: (i) normal direction to the surface, (ii) normal direction to the terrace and (iii) normal direction to the step. There are favourite sites where the probability of reaction is higher than less favourable ones. So the adsorption is not isotropic. This aspect has been analyzed by using molecular beams whose incident angle to the surface is controlled (Groot, 2008).

Here we show the desorption dynamics of  $Pt(533)$  that was exposed to hydrogen background while at a temperature of 150 K for time intervals from

10 seconds to 6 minutes. The integral of each of the curves is proportional to the amount of hydrogen that was adsorbed on the surface. Since we observe a continuous increase in the area of the curve, this means that we are well below the saturation coverage. For the first 3 exposures of 10 seconds, 30 seconds and 1 minute we observe a single peak that develops a tail on the lower temperatures with increased exposure. This means that the hydrogen that was adsorbed is now desorbed from sites with similar energetic status. With exposures of longer than 2 minutes we see the development of another peak that is at around 300 K. The first peak ( $\sim 390$  K) corresponds to the hydrogen that was adsorbed on the steps, whereas the second peak ( $\sim 300$  K) corresponds to the hydrogen adsorbed on the terraces. Ability to prepare a surface that is composed of regions that are very chemically active and other regions that are less chemically active is very useful in catalysis as different requirements are considered for very

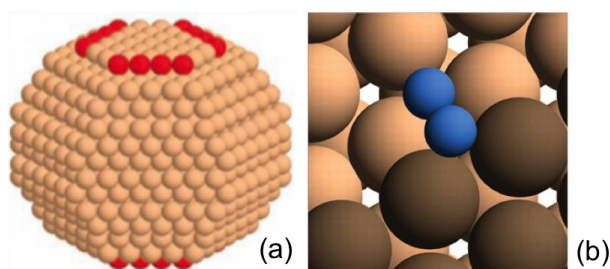


**Fig. 7** TPD from Pt(533) for different hydrogen exposures. Hydrogen desorbing from the terrace has a peak at around 300K, whereas hydrogen desorbing from the (100) step site has a peak at around 390K.

complex reactions. We can consider the ammonia production as an example. Reactions that have high barrier, such as the  $N_2$  dissociation, may occur on the steps and the reactions with a much smaller barrier may occur on the terraces. The step density of the surface can be determined by the rate of the reactions on the steps and on the terrace. This is another reason that has brought theorists into the modeling and analysis of stepped surfaces (Kolb et al., 2014).

The higher reactivity of the steps when compared to the terrace was known and reported very early in the literature (Ehrlich, 1988). More recently McCormack et al. utilizing classical trajectory calculations for the adsorption of hydrogen on Pt(211) proposed the following mechanism: i) direct activated mechanism at terrace site, ii) direct nonactivated mechanism at steps, and iii) indirect nonactivated mechanism at steps (McCormack, 2005). Pt(211) and Pt(533) have the same step type. Groot et al. fitted their experimental results for  $H_2$  dissociation on large Pt(211) and Pt(533) with the model containing the three contributions proposed by McCormack and reasonable agreement was observed (Groot et al., 2008, Groot et al., 2011).

Prediction on the morphology of the nanoparticles for specific reactions have been reported in the literature (Honkala et al., 2005, Hellman et al., 2006). Honkala et al. calculated the rate of ammonia synthesis on ruthenium nanoparticles by using density theory and compared with experimental results and reported that the two approaches agree within a factor of 3 to 20.



**Fig. 8** (a) Ruthenium nanoparticle with the active atoms that are part of the B<sub>5</sub> site. (b) B<sub>5</sub> site on HCP ruthenium (adapted from Honkala, 2005)

They even identified the best possible site on ruthenium that has the minimal energetic barrier for the  $N_2$  dissociation that is the rate-limiting step in the reaction. This site is called the B<sub>5</sub> site and enables that one N atom lands on the top of the step and the other N atom is on the three-fold hollow site at the bottom of the step. This magical site that could be found with a wide range of densities on a nanoparticle cannot be analyzed experimentally. Employing a large stepped surface that contains the B<sub>5</sub> site with a known distribution, to measure the  $N_2$  dissociation may be a possible way to do this analysis.

One more advanced approach is to use a cylindrical crystal for the analysis of a large number of surface orientations at once. Arlow used cylindrical crystals to analyze the adsorption and desorption of molecules from a series of orientations (Arlow and Woodruff, 1985). More recently Hahn et al. started

using them for similar purpose and also including multiple molecular beams (Hahn et al., 2012).

## 5. CONCLUSIONS

The surfaces have been studied extensively and a good understanding of theirs at the atomic level would greatly help in the preparation of well-structured selective catalyzers. The development of experimental techniques and the theoretical simulation encourages the researchers that with continuous effort we can predict the behaviour and then design the next generation of catalysts. Many reactions that are essential in our lives occur on nanoparticles that we cannot analyse. The use of large samples that are structured in regular patterns down to few nanometers can help to get a better understanding of the nanoparticles and the most active sites on them. The improvement of computational techniques and their improving rate of agreement with experiments promises hope in the theoretical scanning of many nanomaterials at a faster rate.

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## NANO-BASED WEARABLE TEXTILE SENSORS: A REVIEW ON LATEST DEVELOPMENTS

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### ABSTRACT

There has been an increase in the customer demand regarding the improvement of the functionality of traditional textiles which are being used nowadays. This demand has directed researchers toward introduction of novel methods and synthesis of new materials, which have improved the properties of the existing textiles. Based on the latest achievements, new terms like ‘smart textiles’, ‘intelligent textiles’, ‘wearable textiles’ and ‘wearable sensors or electronics’ have been introduced into the textile industry in the last years. The smartness of the textiles is realized either by incorporating some active materials into the fibrous structure of by modifying the surface of the textiles in order to give specific characteristics to the textile. Thus, materials which have the ability to sense and possibly react against a certain stimuli in a predetermined way should be integrated into these smart textiles. Manipulation at nanometer level has resulted in the improvement of textile functions such as self-cleaning, actuating, antibacterial and antistatic, UV blocking, communicating and sensing abilities of the textile. Due to these new functionalities, they have found a wide range of applications especially in healthcare, defense systems, sports, and fashion as well. This paper will review the latest developments in smart textiles’ area and especially focus on nanomaterial-integrated wearable textile sensors, the new added properties and their uses.

**Keywords:** smart textiles, wearable textiles, sensors, nanomaterials.

### 1. INTRODUCTION

Textiles are among the oldest materials that are known to humankind. But evolution in technology has made it possible to apply different materials and to integrate electronic devices into these textiles in order to make them smart or intelligent (Meoli, 2002; Kazani, 2012). The 1960s marked an era of innovations in materials with the discovery of shape memory materials. One decade later, in the 1970s, smart materials were introduced with the invention of polymeric gels. Later on in the 1990s, researchers started to integrate these



smart materials into textiles, consequently creating smart textiles and wearable electronics.

By definition a 'Smart Textile' can be any textile which can sense and react to either environmental conditions (ex. mechanical, thermal, magnetic, chemical or other sources) or external stimuli in a predetermined way. They are textiles designed to provide the wearer with an increased functionality (Syduzaman *et al.*, 2015). Regarding their way of production, smart textiles can be categorized in three groups as follows:

- *Passive smart textiles* which are just sensors and can sense only the environmental surroundings.

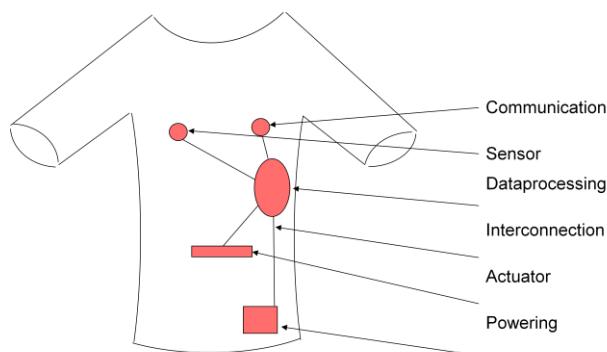
- *Active smart textiles*, which are both sensors and actuators. They can sense the stimuli from the environment and react to these stimuli.

- *Very smart textiles* can sense and react in such a way that they can adapt their performance to the environment conditions and stimuli (Kazani, 2012; Syduzaman *et al.*, 2015; Van Langenhove, 2010; Xiang *et al.*, 2001; Ghahremani *et al.*, 2016; Shyamkumar *et al.*, 2014). They can be shape memory, water-resistant, heat storage, vapour permeable, thermo regulatory etc. They are produced as a result of combinations of traditional textile technologies with other science branches such as material science, electronics, artificial intelligence, biology, mechanics of materials etc.

Today, terms such as 'Smart Textiles', 'Intelligent Textiles' and 'Wearable Electronics' 'Wearable Textiles' are commonly used in every textile-related environment. All these terms refer to textiles that are able to sense stimuli from the environment around them and react and/or adapt to these stimuli (Langenhove *et al.*, 2010; Tao, 2001; CEN/TC, 2011).

### **Smart Textiles System**

Smart textiles are systems composed of different materials and components such as sensors, processors, actuators, power supply and communication (Figure 1). *Sensors* are able to measure parameters e.g. from the human body or the environment and collect data such as temperature, heart beat rate, electrical activity, humidity, pH. The information provided by them is in form of an electrical signal. Many research groups have worked on textile sensors and their improvement (Langenhove *et al.*, 2010; Roh, 2014; Roh, 2016; Xu, 2013; Baldoli *et al.*, 2016; Drabik *et al.*, 2013; Kazani *et al.*, 2016; Kazani *et al.*, 2014).



**Figure 1.** Components of a smart textile system (Kazani, 2012).

*Processors* are used when active processing is required in order to process all the data collected from the sensors. This is achieved with electronic devices as a textile processor does not exist yet.

*Actuators* are able to respond or react to the signals coming from sensors or processors. They can react in the form of a movement, auditive or a visual alarm or in the form of substance release to warn the wearer when something goes wrong.

In order to make the system work, a *Power supply* is required for the smart textile system. It should be combined with energy storage to be able to function as a stand-alone unit. Battery is the simplest example for this aim. Nowadays very thin, flexible and rechargeable batteries suitable for smart textile applications can be found in a printed version ([www.nec.co.jp](http://www.nec.co.jp) ; Bhattacharya *et. al.*, 2009).

*Communication* for smart textiles can be:

- Between the wearer and the suit,
- From the suit to the environment

Communication with the wearer has been realized by France Telecom with an Optical Fibre Flexible Display (Delfin *et. al.*, 2002). Another communication textile based on photonics is that from the Philips Lumalive (<http://www.lumalive.com>).

For communication over longer distances wireless networks can be used. Now this communication is available through textile antennas which can easily be integrated into garments (Hertleer *et. al.*, 2008; Shin *et. al.*, 2009; Vallozzi *et. al.*, 2010).

*Interconnections* are used to join the distinct components within the smart textile. They can be electroconductive yarns woven, knitted, sewn or embroidered in/onto the garment. Another way to achieve them is by screen

printing conductive inks onto the textile surface. Within the suit, communication can be realized by optical fibres or conductive yarns.

In order to have these components present in textiles a special property is required, namely electroconductivity to transport electrical current. Thus, it is necessary to develop electroconductive textiles, which can be obtained in several ways, for example by integrating conductive fibres or yarns, by applying conductive coatings, or by using conductive inks or conductive polymers (Xue *et. al.*, 2007; Gasana *et. al.*, 2006; Banaszczyk *et. al.*, 2009).

This paper will review the latest developments in smart textiles area and especially focus on nanomaterial-integrated wearable textile sensors, the new added properties and their main application areas.

### **Processes and materials used in smart textiles' production**

To manufacture these wearable textile systems, electroconductive textiles are needed. Electroconductive textiles are materials which can conduct an electrical current (CEN/TC 248/WG 31 Technical Report) and they can be achieved by using conductive fibres, yarns, coatings, polymers or inks (Kazani, 2012; Xue *et. al.*, 2007; Banaszczyk *et. al.*, 2009; Lim *et. al.*, 2010; Ghahremani *et. al.*, 2016).

Electroconductive fibres/yarns can be divided in two main groups:

- Fibres/yarns, which are naturally conductive. They can be pure metals mainly silver, copper and stainless steel, produced by methods such as wire drawing, bundled wire drawing, cutting production, melt spinning and melt extraction.
- Fibres/yarns treated to be conductive. These fibres/yarns are coated with metals, galvanic substances or metallic salts. The coating processes used are: electroless plating, evaporative deposition, sputtering and coating with conductive polymers.

There are several techniques that integrate conductive fibres/yarns into a textile structure such as knitting, weaving, embroidering, sewing and needle felting. Conductive textiles can be obtained also by treating the surface of the traditional textile. This can be achieved by coating with a conductive layer, by coating with polymers such as polypyrrole (Banaszczyk *et. al.*, 2009), deposited onto the surface of textile substrates, by printing with conductive inks, 3-D screen printing (Kazani, 2012), ink jet printing, and microencapsulation as well.

Other processes used to produce smart textiles are fiber spinning, melt blowing, electrospinning, wet spinning, electrocentrifuge spinning, force spinning, and photolithography (Kazani, 2012; Krucinska *et. al.*, 2013; Krucinska *et. al.*, 2016; Mottaghitalab *et. al.*, 2006; Kazani *et. al.*, 2016; Shin *et. al.*, 2009; Filipowska *et. al.*, 2016; He *et. al.*, 2012; Valipouri *et. al.*, 2015;

Yanilmaz *et al.*, 2014; Ghahremani *et al.*, 2016a; O'Haire *et al.*, 2014; Ghahremani *et al.*, 2016b; Kizildag *et al.*, 2016a and b).

Regarding materials that have been used to design smart textiles, the most commonly added ones are *electrically conductive polymers*. Polymeric or carbon coated threads, conductive yarn, conductive rubber, conductive ink have been developed into sensors or used as interconnection substrates (Syduzzaman *et al.*, 2015). Electrically conducting nanoadditives also have been added to composite materials to produce functionalized composites (Krucinska *et al.*, 2013; Gasana *et al.*, 2006; Krucinska *et al.*, 2016; Meoli, 2002; Kazani *et al.*, 2016; Kazani, 2012; Kazani *et al.*, 2012). Electrospun nanofibers, nanotube-based fibers and yarns and polymer nanocomposites as well have been used for various textile applications (Rostami *et al.*, 2015; Li *et al.*, 2014; Thilagavathi *et al.*, 2008; Lee *et al.*, 2007). Electrospun multilayer polycaprolactone (PCL) and polyamide 6 (PA) nanofiber mats incorporated with pH sensitive dyes revealed their potential to be applied in protective clothing as chemical and warfare detection in the gas form (Rostami *et al.*, 2015). The potential of electrospun polyurethane (PU) nonwoven mats for improving protective properties of clothing used by agricultural workers was studied by Lee *et al.*, (2016). No significant change was observed for moisture vapor transport; however, it showed the highest air permeability compared to most of the protective clothing materials used nowadays (Lee *et al.*, 2007).

*Optical fibers* can also be utilized due to their ability to transmit light for optical sensing, detect fabric deformations, and perform chemical sensing (Krucinska *et al.*, 2016; Gauvreau *et al.*, 2008; Delfin *et al.*, 2002). Some other materials used for electrical textile properties improvement are carbon-based materials (ex. carbon nanofibers, carbon black nanoparticles, and carbon nanotubes), clay nanoparticles and metal oxide nanoparticles (ex.  $\text{TiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{MgO}$ ,  $\text{ZnO}$ ) (Wallace *et al.*, 2007; Mottaghitalab *et al.*, 2006; Kizildag *et al.*, 2016a and b; Atayev *et al.*, 2014; Ashayer-Soltani *et al.*, 2016; Sklyar *et al.*, 2012).

Moreover, *inherently conducting polymers* (ICPs) are used because of their actuator-like behavior and sensing properties. The most well known and used ones are polypyrrole, polyaniline, polyacetylene, polythiophene (Coyle *et al.*, 2007; Kizildag *et al.*, 2016b; Mottaghitalab *et al.*, 2006). Among these, polypyrrole (PPy) is highly preferred due to its high elastic behavior and mechanical properties and also its high air stability. Continuous wet spinning can be used to fabricate ICP-based textile materials (Coyle *et al.*, 2007). These material types have a drawback in that their resistivity and high response time may change in time (Syduzzaman *et al.*, 2015). The mechanical and electrical properties of ICPs have been improved more by adding carbon nanotubes (CNTs) into the structure (Kizildag *et al.*, 2016b).

An increase of 50% in ultimate tensile strength and 120% in elastic modulus of PANi-CNT composite fibers was observed recently by Mottaghitalab *et. al.*, (2006).

*Shape memory materials* are another category of materials used in textile industry. The most common used ones are Ni-Ti and Cu-Zn alloys which have provided increased protection against heat. Based on the behaviors of these types of materials, a new class of compatible materials has been developed (Wang *et. al.*, 2004). These are called electroactive polymers (EAPs) and have found a wide range of applications (Syduzzaman *et. al.*, 2015).

A fourth group of materials are *phase change materials* (PCMs), which have the ability to store heat when needed and release it if it overcomes the normal range. They can be very useful in the production of protective garments in all seasons. The main issue to be considered with these materials is that their temperature range should be within human skin temperature standards (Syduzzaman *et. al.*, 2015).

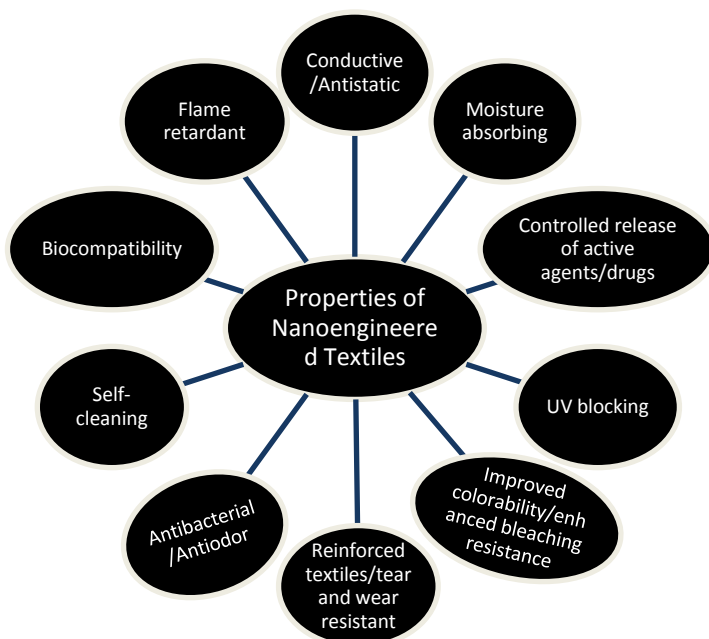
Materials with color changing characteristics upon external conditions stimuli, called *chromic materials* (CMs), are also an interesting group of materials to be studied. They find a wide range of applications especially in fashion design. There are materials sensitive to light, heat, liquids or gases, electricity and pressure (Rostami *et. al.*, 2015) and they are categorized as photo, thermo, electro, piezo and solvate-chromic materials.

Recently coating of existing textiles with various nanoparticles, which imparts some important characteristics to the materials such as antistatic, UV resistant, antibacterial and self cleaning, has been realized. In addition, polymer nanocomposites composed of a polymer dispersed with nanoparticles or nanofibers have been widely studied (Gowri *et. al.*, 2010). More details about these types of applications and the new properties introduced to the traditional textiles will be given in the section below.

### **Nanomaterial-integrated wearable textiles: added properties and their application areas**

A very interesting and promising area is the incorporation of nanomaterials into traditional textile industry. There has been a great interest of researchers toward the production of textiles with specific intelligent properties such as materials with high electrical or conductive properties, light weight, high flexibility, and if possible low price textiles (Lim *et. al.*, 2010; Sawhney *et. al.*, 2008; Thilagavathi *et. al.*, 2008). Due to nanotechnology the existing traditional textile properties have been improved and new concepts like '*nanoengineered functional textiles*' have been introduced to the field. They are textiles or textile products where nanotechnology is used during a production or finishing step. Some of the introduced properties to these textiles are hydrophobicity, biocompatibility, antistatic and antiwrinkle

behavior, antibacterial ability, light guidance, UV blocking, and sensing capacity of the material (Yetisen *et. al.*, 2016). Other properties of the fabric also such as fabric softness, breathability, high tensile strength, conductivity, abrasion stability and fire retardancy have been improved (Gowri *et. al.*, 2010). The following *Figure 2* shows the possible functions of nanoengineered textiles.



**Figure 2.** Possible functions of nanoengineered textiles.

Some of the traditional textile industry polymers such as polyester and nylon are well known for their static properties due to their hydrophilicity. Nanoparticles (NPs) have been added to the fabric to increase the textiles' antistatic properties. ZnO NPs were added to polyester fabrics and immobilized to obtain fabrics with better antistatic properties (Zhang *et. al.*, 2009). In another study, SnO<sub>2</sub> particles were added to polyacrylonitrile fibers to introduce electroconductive channels with antistatic properties (Wang *et. al.*, 2004). Silver NPs also have been utilized to improve the static properties of the textile (Ghahremani *et. al.*, 2016a and b). Moreover, inspired by Lotus effect, textiles with water and/or oil repellence properties have been produced by researchers. The fabric is either coated with NPs or additives can be added on its surface (Qiaozhen, 2007).

Light weight and strength of fabrics are two other important properties that need to be imparted to the wearable technology. This has been achieved by reinforcing polymers with CNTs (Kizildag *et. al.*, 2016a and b; Liu *et. al.*, 2016; He *et. al.*, 2012; Gasana *et. al.*, 2006). Liu and other colleagues (2016) produced CNT/epoxy composites in order to enhance the mechanical and electrical properties of the material. The toughness of the CNT buckypaper was increased by 14-fold and mechanical and electrical properties were significantly increased (by 465 and 168 %, respectively) compared to the pure resin (Liu *et. al.*, 2016). Another characteristic studied by researchers is the UV blocking ability of the fabric (Chien *et. al.*, 2003; Gowri *et. al.*, 2010). It has been proven that both ZnO nanorod incorporation into cotton or coating its surface with TiO<sub>2</sub> increases the UV protective ability of the cotton-based fabric (Saito, 1993; Xin *et. al.*, 2004; Yang *et. al.*, 2004). Furthermore, antibacterial and fungicidal textiles are of utmost importance for the wearers. Most commonly used materials to introduce this property to the textiles are TiO<sub>2</sub>, ZnO and Ag. For example, Ag NPs have revealed their ability to react with specific proteins in the cell and thus adversely affect cellular function and inhibit its growth. Ag<sup>+</sup> ions bind to sulfur-containing proteins on the cell membrane, thus, change their cell wall. They also suppress the growth and proliferation of fungi and bacteria, reducing in this way itchiness and bad odor (use in socks) (Liu *et. al.*, 2010). PAN functionalized with multi-walled CNTs and silver nitrate showed to be suitable to be used as antistatic and antibacterial material (Kizildag *et. al.*, 2016b). A last studied property of textiles is their wrinkle-resistant characteristic. Again NPs (ex. TiO<sub>2</sub> and SiO<sub>2</sub>) have been introduced to cotton and silk for this aim and they have shown better wrinkle resistance compared to the traditional non-NP containing ones (Chien *et. al.*, 2003; Lu *et. al.*, 2007; Siegfried *et. al.*, 2007). Furthermore, textiles which can act as carriers of drugs or other active materials in order to have a controlled release behavior have been studied and produced. For example, antibacterial fabrics which can release in a controllable fashion a biocidal agent can be produced. The following table describes some of the most commonly used nanomaterials together with the specific textile material to produce textiles with various desired properties (see Table 1).

**Table 1.** Some nanomaterials and the specific textile material used to produce textiles with various improved properties (adapted from Siegfried *et al.*, 2007).

Nanomaterial	Textile material	Property added to the textile
Cu Carbon Black CNT Polypyrrole Polyaniline	PP/PA PU PP, PA Viscose/wool Polyester	Increased conductivity
CNT SiO <sub>2</sub> TiO <sub>2</sub> Fuoroacrylate-NP	Cotton Cotton, viscose Cotton, wool/PA, Polyester Polyester, PA	Water and oil repellent (self cleaning/antiadhesive)
Ag TiO <sub>2</sub> Ag + TiO <sub>2</sub> ZnO Chitosan/polybutylacrylate CNT Chitosan	Polyester, cotton, silk, PA, PP Cotton, cellulose fibers Cotton Cotton PAN	Antibacterial/antiodor
CNT ZnO Al <sub>2</sub> O <sub>3</sub> SiO <sub>2</sub>	PVA, PS, PP Cotton  Polyester	Reinforced textiles
CB (carbon black) Nanoporous hydrocarbon coating	Cotton, polyamide Polyester	Improved colorability
ZnO TiO <sub>2</sub>	Cotton Cotton	UV blocking
Sb <sub>3</sub> O <sub>2</sub> Montmorillonite CNT Borosiloxane	Pu, PP, PA, cotton Ethylene vilyl acetate PP	Flame retardandy

### Nano-based wearable textile sensors and their applications

Nanotechnology is applied on the textile sensors or so called 'textrods' as well, which are used mainly in the military, healthcare and sport field for monitoring of vital signs and the position or moves of the body, measurement of different parameters such as temperature, heart beat, digestive systems, joints, ultrasounds, sweat, etc. (Langhenove *et al.*, 2010; Thilagavathi *et al.*,



2008). Furthermore, pressure, humidity and temperature sensors have been introduced to textile technology (Drabik *et al.*, 2013; Lim *et al.*, 2010). Lim *et al.* (2010) produced a multifunctional cotton-based conductive sensor which showed excellent gas and optical properties at room temperature. They could grow ZnO nanorods radially oriented and densely onto the surface of cotton fabrics and the nanorods were highly resistant toward stress and washing cycles.

Materials science in combination with digital communication has developed a lot in the last decades and this has resulted in the improvement of chemical and biochemical sensors' functions. There is a high demand of wearable sensors to be used in kinematic analysis, monitoring of vital signs and biochemical analysis (Wallace *et al.*, 2007; Shishoo *et al.*, 2005; Hannon *et al.*, 2014). Sweat detection sensors, sweat electrolyte sensors, oxygen sensors, glucose sensors, saliva-based sensors and other sensor types have been developed recently. Most of them are sensors used to sense or monitor samples from the body.

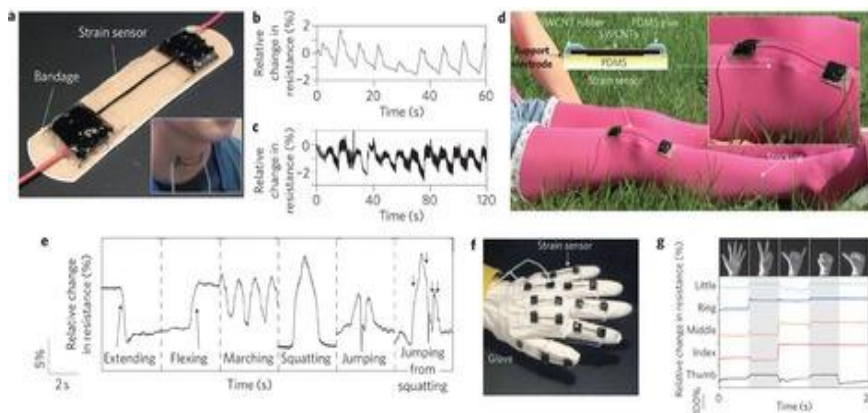
An important issue is to be able to monitor substances which are present in the environment we live and if possible warn the person about any possible hazard. This can be achieved by wearable chemical sensors due to their ability to sense hazardous materials, which can be very helpful to people working in places dangerous for their health. In a recent study, nonwoven fabrics prepared by melt-blowing and electrospinning methods were tested for their sensory capacity against chemical vapors such as methanol acetone, benzene and toluene, which are substances extensively used in industry and are highly toxic. All of these different polymer-based fabric sensors combined with CNTs showed good electrical conductivity, high sensitivity and they changed their resistance in the presence of chemical vapors (Krucinska *et al.*, 2016b). These types of sensors should be designed so that they are placed on the body in specific positions for a long period of time and they are named as gas sensors. It is crucial as well to choose an appropriate fabric with suitable permeability to design gas sensors.

Microneedle technology is another method used in wearable biochemical sensors. Highly biocompatible devices or patches are needed for this aim since there is a direct contact with the skin. Simultaneous detection of some analytes in a tissue environment was studied by Miller *et al.*, who showed the microneedle potential to measure pH change, and level of glucose and lactate as well (Miller *et al.*, 2012). These types of devices can be utilized for transdermal drug delivery applications also; however, their fabrication is quite challenging.

Nowadays, microfluidics has a great role in developing personalized healthcare or point-of-care sensing devices (Coyle *et al.*, 2014). In the context of EU BIOTEX project, Curto and other colleagues (2009) developed

a flexible microfluidic system that can sense the sweat pH (Morris *et. al.*, 2009). These sensor types at the moment are single-use ones and still the problems related to their multiple use and long term usage need to be considered.

Soft sensing technologies have been developed by combining electronics, fabrics and host garments. They are named ‘wearable robots’ and are based on soft materials. Menguc and his colleagues (2014) studied the design and characterization of a soft sensing suit in order to monitor the movement of knee, hip and ankle sagittal at various angles by means of hyper-elastic strain sensors integrated into a soft wearable garment. The authors claim the ability of the wearable sensor to be used as single-piece garments and also they could help medical doctors follow the daily motion of their elderly patients. In another study, a stretchable carbon nanotube strain sensor for human-motion detection was developed by Yamada *et. al.*, (2011). They could assemble the carbon-nanotube sensors on stockings, bandages and gloves in order to produce devices that can detect different types of human motion, including movement, typing, breathing and speech (*Figure 3*). For this aim they fabricated aligned thin films of single-walled CNTs. The authors report that their mechanism has a high durability, fast response and low creep and a strain measuring capability of 50 times higher than the conventional metal strain gauges (Yamada *et. al.*, 2011).

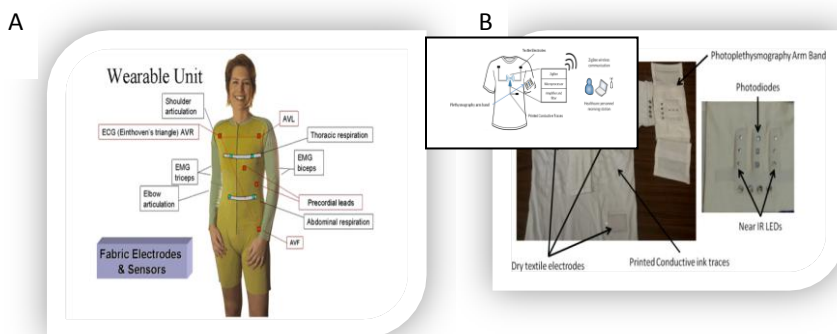


**Figure 3.** Stretchable wearable devices (Yamada *et. al.*, 2011).

Wearable chemical and biochemical sensor types can be classified as wearable colorimetric and electrochemical sensors. The former undergoes a color change whereas the latter involves the use of electrodes that can measure electrochemical changes as a result of the interaction of the chemical with the surface of the sensor. Novel wearable electrochemical sensors have

been produced by means of various conductive polymers and carbon-based nanomaterials (Coyle *et. al.*, 2014; Guinovart *et. al.*, 2013; Wallace *et. al.*, 2007). It should be emphasized that even though various types of wearable chemical sensors have been produced lately, a lot of work is needed in terms of optimizing most of them for their routine usage.

Monitoring the patients having chronic illnesses is of great importance. Wearable devices, including sensors, electrodes or other connections in them, can make possible the continuous monitoring of physiological signals in real time (see Fig. 4A). For instance, a wearable textile sensor was produced recently for monitoring cardiovascular diseases (Wallace *et. al.*, 2007). Carbon-loaded elastomer strain sensors and fabric bioelectrodes were integrated into a cotton/Lycra<sup>®</sup> shirt to measure body position, movement, respiration and functions like electrocardiogram and electromyogram (Wearable Health Care System = WEALTHY EU project). A MARSIAN smart glove with noninvasive sensors in it can measure skin temperature, its potential and skin electrical conductance as well. In another study, Shyamkumar *et. al.*, (2014) produced wearable textile systems that could be used for patients diagnosed with cardiovascular diseases. The authors mention the e-bra and e-bro systems designed for women and men, respectively (Fig. 4B).



**Figure 4.** (A) An e-textile system for remote, continuous monitoring of physiological and movement data. The sensors can record both electrocardiographic and electromyographic data (Courtesy of Smartex, Italy) (Patel *et. al.*, 2012) and (B) E-bro and the photoplethysmography arm band (Rai *et. al.*, 2014).

These systems are multichannel wearable wireless textile-based nanosensors designed to monitor blood pressure and ECG (Shyamkumar *et. al.*, 2014). Similar to this sensor type other sensor systems that can measure temperature, blood oxygen level and respiration can be introduced into the wearable textiles.

Wearable textiles have found a wide range of application in defense systems as well namely, textiles for smart uniforms (able to monitor specific characteristics), ballistic protection, chemical and biological protection and sensor and energy storage fabric production (Lee *et. al.*, 2007; Wallace *et. al.*, 2007; Thilagavathi *et. al.*, 2008). They are also used for applications such as heating, monitoring of human vital signs or illuminating textiles.

## 2. CONCLUSION

Combination of traditional textile technologies with other science branches such as material science, electronics, artificial intelligence, biology, chemistry and mechanics of materials has resulted in the production of new textiles in the last years. Textiles that can sense and react to either environmental conditions or other external stimuli in a predetermined are called 'Smart textiles'. They have been designed mostly to provide the wearer with an increased functionality. Smart textiles are categorized as passive, active and very smart textiles. While the passive ones can only sense the stimuli, the active textiles can sense and react to the stimuli and the very smart ones can sense and react in such a way that they adapt themselves according to the stimuli. Smart textiles for applications starting from military, fire-fighters and first responders to medical garments, healthcare, sport wearings and also for fashion design and casual garments have been studied by many researchers. Lastly, research on the improvement of the properties and the functionalities of textiles is extensively being undertaken. Integration of nanomaterials (nanoparticles, nanofibers, nanofillers) into the traditional textile materials has resulted in significant improvements. This is achieved by incorporating nanomaterials inside the fibrous structure of the textile or by modifying its surface by coating with electroconductive materials.

Wearable devices, including sensors, electrodes or other connections in them, are among the most interesting applications of textile technology. They make possible the continuous monitoring of physiological signals in real time. There is a high demand of wearable sensors to be used especially in kinematic analysis, monitoring of vital signs and biochemical analysis. For instance, sweat detection sensors, sweat electrolyte sensors, oxygen sensors, glucose sensors, saliva-based sensors have been developed recently. Most of them are sensors used to sense or monitor samples from the body. Moreover, gas sensors able to monitor substances which are present in the environment we live and if possible warn the person about any possible hazard are produced. For this aim, various conductive polymers and carbon-based nanomaterials have been used. However, it should be emphasized that even though various types of wearable sensors have been produced, a lot of work is needed in terms of optimizing most of them for their routine usage.

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## NANOCAGES AND NANOTUBES: ORGANIC/INORGANIC HYBRIDS IN BIOSENSORS

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### ABSTRACT

In this review article we will discuss new opportunities that are emerging from the combination of organic and inorganic building blocks at the nanoscale. The combination of organic and inorganic nanostructures into nanocomposites is called nanohybrids. Studies in colloidal materials, self-assembly, and polymer-based nanostructuring are coming together to form these novel materials. Gold nanocages and nanoboxes are examples of the novel structures created to combine organic and inorganic molecules. The distinctive properties of nanomaterials come from their structures at very small dimensions. Nanocages are hollow structures with porous walls, and nanoboxes are hollow structures with nonporous walls (Skrabalak *et al.*, 2007). Gold nanocages provide unique optoelectronic properties. Surface plasmon resonance is an optical property resulting from the vibration of conduction band electrons. Gold nanocages and OCT can also be used in an encapsulation technique, where magnetic nanoparticles are encapsulated within the nanostructure for field-directed delivery.

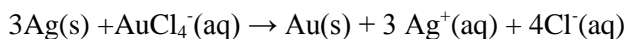
Keywords: nanocages, nanotubes, organic/inorganic hybrids, biosensors

### 1. INTRODUCTION

As will be shown in this review article, new opportunities are emerging from the combination of organic and inorganic building blocks at the nanoscale. The combination of organic and inorganic nanostructures into nanocomposites is called nanohybrids. Studies in colloidal materials, self-assembly, and polymer-based nanostructuring are coming together to form these novel materials. Recent research initiatives are designing nanoparticle conjugates of organic/inorganic molecules which enable the coupling of the intrinsic functionalities of these diverse molecular systems with the size and shape dependent optical and electronic properties of nanomaterials. Specific

nanomaterials show an inclination for combining with organic and biological molecules, and their inherent properties prove to be beneficial and unique to their nanostructure. The properties of hybrid nanomaterials make them exemplary options for utilization as biomedical applications because of their combined organic and inorganic properties. Inorganic nanomaterials have special optoelectronic properties that can be combined with the biological reactivity of organic molecules. Current nanohybrids are giving promising futures to biosensors applied for *in vivo* optical imaging and environmental compound detection.

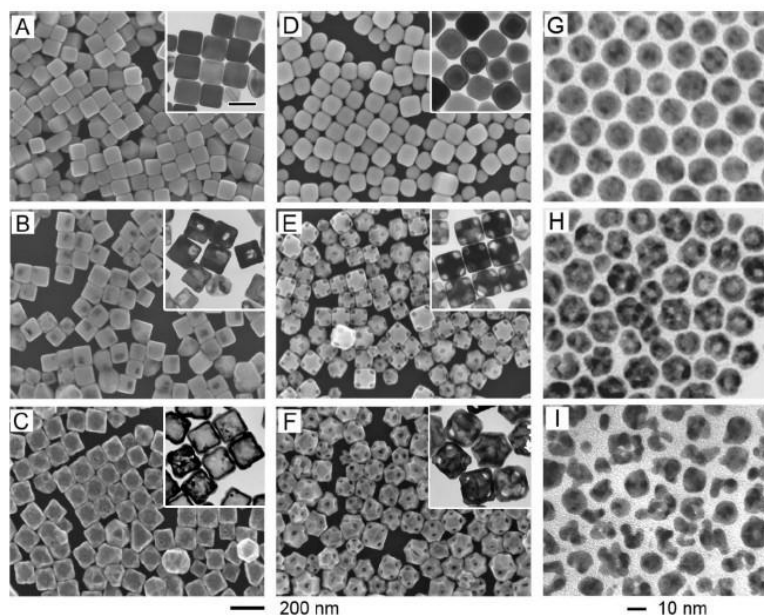
Gold nanocages and nanoboxes are examples of the novel structures created to combine organic and inorganic molecules. The distinctive properties of nanomaterials come from their structures at very small dimensions. Nanocages are hollow structures with porous walls, and nanoboxes are hollow structures with nonporous walls (Skrabalak *et al.*, 2007). Like carbon nanotubes, the gold nanocages can be used as a housing or carrier site for organic molecules, but unlike carbon based products, gold is biologically inert and safer to implant into the body. The gold nanocages can be prepared through a simple synthesis technique based on the galvanic replacement reaction between silver (Ag) nanocubes and gold chloride (HAuCl<sub>4</sub>). The replacement reaction takes place in aqueous solution and is as follows:



The silver nanocubes used in the galvanic reaction are synthesized via polyol synthesis. AgNO<sub>3</sub> is reduced with ethylene glycol to generate silver atoms and then promote silver crystals. The defined nanocube structures are synthesized by the addition of polyvinyl pyrrolidone (PVP). PVP selectively binds to the {100} facets, so silver-nanorods capped by {100} side faces are preferentially passivated by PVP. The {111} sides of the silver-nanorods will continue to grow and construct the nanocubes structure (Skrabalak *et al.*, 2007). By limiting the growth of specific facets, and promoting the growth of others, very specific nanoscale geometries can be formed. The PVP addition acts on the initial crystal seed by stunting a preferred axial growth. Nucleation and subsequent growth is forced to conform to an applicable geometry. Once the silver-nanorods are synthesized, the gold structures can be made by the aforementioned galvanic replacement reaction.

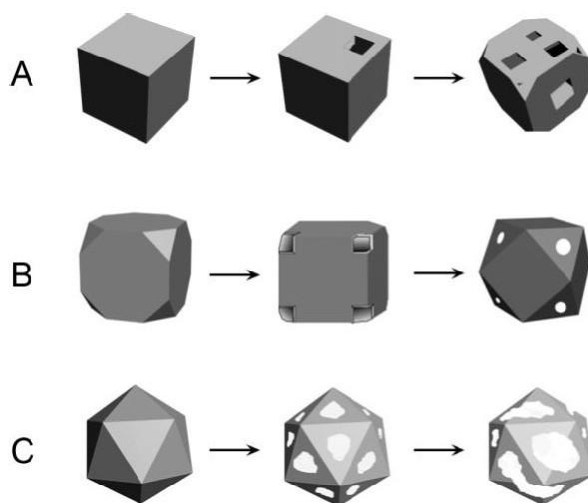
Starting with silver nanocubes gives a controlled morphology for the gold to begin nucleation and growth; moreover, the cubic template imparts its shape to the growing gold structures. Figure 1 shows SEM and TEM images of the galvanic reaction. The images show a series of reactions with different silver-nanocubes geometries. SEM images A-C are silver- nanocubes with

sharp corners. SEM images D-F are silver-nanocubes with truncated corners. Images G-I are TEM pictures of twinned silver-nanocubes. As the silver is replaced in the silver nanocubes, the stoichiometry dictates a loss of mass in the cubes and in turn, hollow cubes form. In Figures 1G, 1H and 1I the hollow structures are very noticeable, and picture 1G shows the precise pore definition at the corners of the gold nanocages.



**Fig. 1.** SEM and TEM Images Showing Galvanic Replacement Reaction of Ag and Au (Skrabalak *et al.*, 2007).

During the galvanic reaction the gold replaces all silver atoms, which returns to aqueous solution. Figure 2 shows a schematic of the transformation of silver-nanocubes into gold nanoboxes or nanocages. The replacement reaction proceeds by silver dissolution at specific sites, formation of nanocages consisting of thin uniform walls due to replacement and gold-silver alloying, and the generation of pores by de-alloying. Just as the geometries of silver-nanocubes could be controlled, the conformation of the pores in gold-nanoboxes can be varied by applying a PVP passivation layer also. When truncated silver-nanocubes are used as a precursor (Image B, Figure 2), the unprotected corners serve as primary sites for silver dissolution and lead to the formation of well-defined pores. By increasing the control over nanoparticle geometries, their functionality can be fine tuned for technological applications.



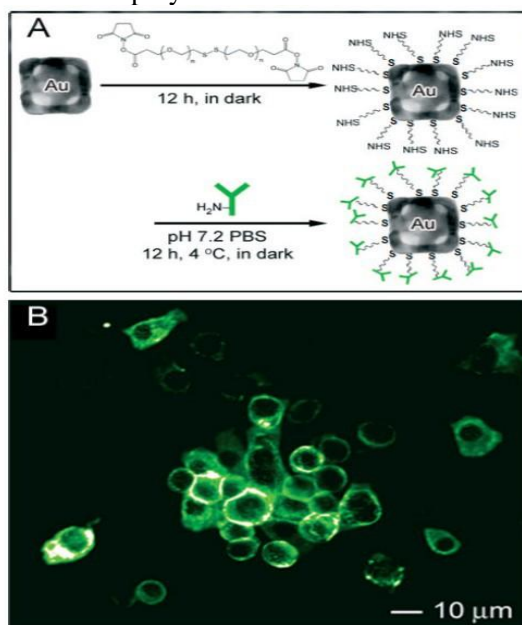
**Figure 2.** Schematic of Different Shape Ag-nanocubes into Au-nanocages (Skrabalak *et al.*, 2007).

Gold nanocages provide unique optoelectronic properties. Surface plasmon resonance is an optical property resulting from the vibration of conduction band electrons. At the resonant frequency, photons can be scattered or absorbed. Surface plasmon resonance is dependent on the scattering or absorption of incident phonons; moreover, the scattering and absorption of photons are dependent upon the size and shape of the nanostructure. Gold-nanocages have a tunable surface plasmon resonance that extends into the near-infrared (700-900 nm). At these wavelengths, the attenuation caused by blood and soft tissue is negligible. Tuning of specific nanocages is done by adjusting the ratio of Ag to H<sub>Au</sub>CL<sub>4</sub> in the galvanic replacement reaction. Studies have produced SPR peaks from 400-1200 nm. Having surface plasmon resonance peaks in the visible region gold-nanocages can be used in sensing and image contrast enhancement. Organic molecules cannot offer comparable properties due to their size and amorphous structure, so using carriers, like gold nanocages, creates hybrids with organic and inorganic properties.

### Application

The novel optical properties of gold nanocages can be exploited by surface functionalization. The surface of gold nanocages can be functionalized with biological targeting molecules like ligands and antibodies. Ligands act within the body's compliment system which assigns functional markers to individual molecules. These markers are then used to dictate movement and interactions

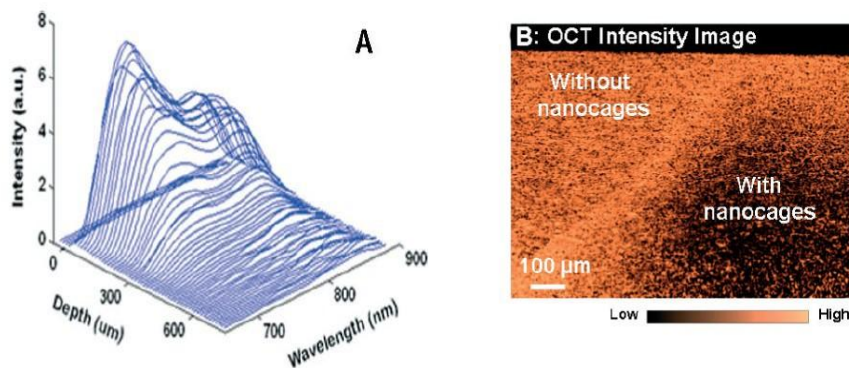
between biological molecules. By attaching ligands to the gold nanocages, the nanocages can interact with and perform a role within biological systems. For example, gold nanocages functionalized with specific antibodies can detect cancerous tumors, so the adherence of organic molecules to the gold surface is essential. Sulfur has a very strong affinity for gold (85 - 145 kJ/mol), so functional groups with sulfur can easily be attached in monolayers to gold nanocages. Organo-sulfur molecules spontaneously assemble into stable, organized layers on gold surfaces. The attached sulfur groups can then be individually functionalized with biological molecules. Biological molecules like small-molecule inhibitors and antibodies can recognize specific receptors in tumor cells or glucose, for use in cancer sensing or diabetic maintenance. The optical properties of the gold nanostructures are used for locating purposes, while the biological reactivity of the functionalized surface groups cause the nanohybrids to migrate to specific sites. Figure 3A shows a schematic illustration of how antibodies bind with the surface of gold nanocages. Figure 3B is a fluorescence image of cells that were treated with antibodies attached to gold nanocages. In Figure 3A, there are two stages to the functionalization of the gold nanocages. The sulfur group of a polymer adsorbs onto the surface of the nanocages, and the active biomolecules bind with the sulfur-based surface polymer.



**Figure 3.** (A) Two-step protocol used to conjugate Au-nanocage surfaces. (B) Fluorescence of Au-nanocages, functionalized with breast cancer marker cells (Skrabalak *et al.*, 2007).



The resultant application of the nanocages unique optical properties, the fluorescence shown in Figure 3B, is exploited in optical coherence tomography. Optical coherence tomography (OCT) is a diagnostic tool for noninvasive, *in vivo* imaging. Image contrast is produced from the scattering and absorption of light by tissue. To improve image contrast, materials with unique optical properties can be implanted as contrast agents. The near infrared SPR peaks make gold nanocages a viable option in OCT diagnostics. Gold nanocages synthesized to 35 nm with SPR peaks tuned to 700 nm embedded in a gelatin and titania matrix illustrated the unique fluorescence and functionalization of the nanocages. The titania granules and gelatin mimic the scattering of biological tissues, but the functional groups adsorbed to the surface of the nanocages result in attenuation of the fluoresced light.. Figure 5A shows the attenuation of back-scattered light in the gelatin-titania matrix. Figure 5B is an OCT image of tissue that has attenuation from the strong absorption of the Au nanocages. The gold nanocages provide absorption contrast in OCT imaging by creating attenuation due to the functionalized adsorbates.



**Figure 4.** (A) Depth-dependent spectra of the back-scattered light from gelatin-titania matrix (mimicking live tissue). (B) OCT image displaying the strong attenuation from the portion which contains Au-nanocages (Skrabalak *et al.*, 2007).

Gold nanocages and OCT can also be used in an encapsulation technique, where magnetic nanoparticles are encapsulated within the nanostructure for field-directed delivery. Instead of surface functionalized nanostructures, the particles injected into the nanocages direct their movements *in vivo*. OCT can utilize magnetic particles as contrast dyes by collecting back-scattered light, in the same manner as using functionalized surfaces. Magnetic iron oxides, like Fe<sub>3</sub>O<sub>4</sub> nanoparticles, are synthesized into the gold nanocages. These particles are responsive to a magnetic field at lower frequencies than MRI's



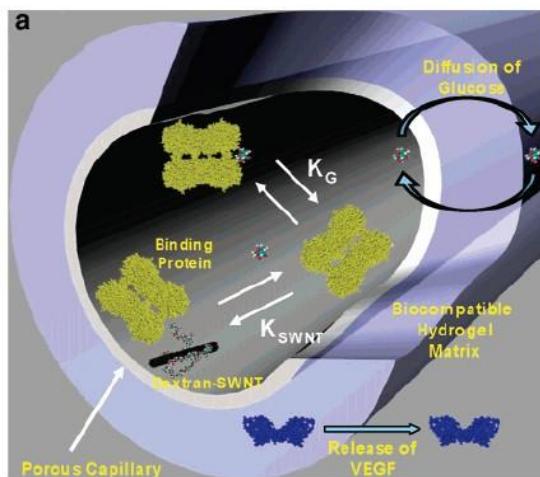
and are modulated within the soft tissue. The mechanical oscillations will produce unique light scattering when combined with OCT (Oldenburg *et al.*, 2006). By comparing the tissue before and after the application of a modulating magnetic field, the nanoparticles can be pinpointed by the resultant light scattering. An early attempt at using magnetic nanoparticles was in tissue scaffolds to locate specific growth sites and recognizing labeled and unlabeled cells. A more recent use of the magnetic nanoparticles is by functionalizing macrophage cells. Macrophages are one of the cells that form during immune responses at wound sites, so locating and accounting for the concentration of macrophages can be used to assess healing and immune response.

Similar to nanocages, functionalized carbon nanotubes are being investigated as substrates for biosensors. Carbon nanotubes, CNT, can be used in a similar fashion to gold nanocages; however, their electrical properties are superior and they are much more readily hybridized with organic components. In single-wall CNT, all atoms are surface atoms and are especially sensitive to surface adsorption. CNT can be used to promote reactions of specific biomolecules like dopamine, norepinephrine and cholesterol, but poor solubility is a problem for the use of CNT in biomedical applications (Dhand *et al.*, (2008)). CNT do not inherently dissolve into aqueous solution; thus, the combination of organic polymers and CNT is a challenge in the fabricating carbon based nanohybrids for biomedical applications.

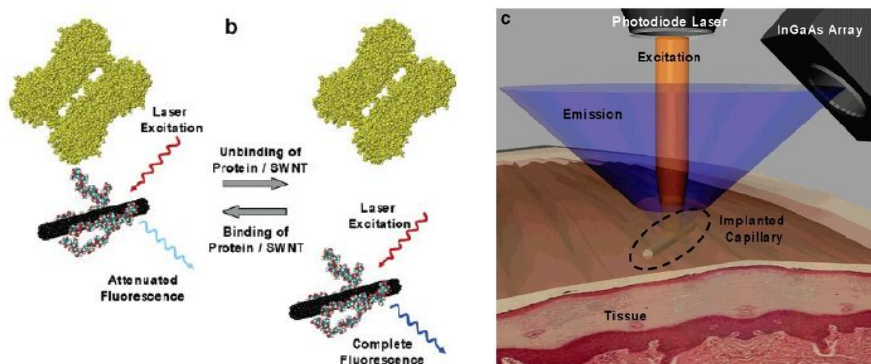
Wrapping of CNT with polymeric chains can be used to improve solubility without hindering their mechanical or electrical properties. Polyaniline is a polymer that readily adheres to carbon nanotubes and is conductive. It is used in Schottky devices and chemical sensors; however, for biosensors, the polymer has to be electrochemically active and conducting in neutral solutions, this promotes coupling to a biological system. Polyaniline is not active in neutral solutions, so it can be doped with CNT and other polymers to create electrochemical activity and conduction at neutral pHs (Dhand *et al.*, 2008). The fabricated, coated CNT can be used in a variety of biosensor applications because its solubility and chemical reactivity make it a viable candidate for implantable sensors.

A recent development in implantable biosensors is using functionalized CNT to detect glucose levels for diabetic maintenance. Like the gold nanocages, CNT have near infrared fluorescence and can be used as tissue implantable biosensors. Fluorophore-based optical sensors use protein binding and functionalization of the nanostructures' surface. The protein or other organic molecule binding to the surface induces fluorescence attenuation, and this produces an intensity reduction of the back-scattered light. In such a biosensor, the CNT would be functionalized with an analyte derivative. The analyte, desired measured substance, is glucose for blood sugar

concentrations. In the case of glucose, the analyte derivative could be a series of sugars, *e.g.* dextran. The analyte derivative reacts with the binding protein, glucose oxidase. As the targeted protein is introduced to the nanotubes' surface, the equilibrium is shifted and the fluorescence attenuation occurs (Barone *et al.*, 2005). The functionalized nanotubes are placed inside a capillary which allows glucose diffusion. For biocompatibility, the capillary is implanted with a hydrogel coating, to reject encapsulation. As the glucose flows through the capillary and reacts with the CNT surface, the shifting of fluorescent intensity can be measured and intensity variation signals change in glucose concentrations. Figures 5A-5C are a schematic of the diabetic monitoring capillary. Notice in Figure 5B, the binding of the protein and CNT is a reversible process; therefore, the CNT does not act like an expendable reagent. The sensors lifetime is not dependent upon surface area because the proteins cycle through. Unlike current glucose testing which involves incessant blood tests, an implantable CNT-hybrid device just employs a direct, incident light to examine glucose levels. Gold and carbon nanostructures provide highly biocompatible materials for the substrates of similar implantable medical devices.



**Figure 5A.** Schematic of glucose-monitoring capillary(Barone *et al.*, 2005).



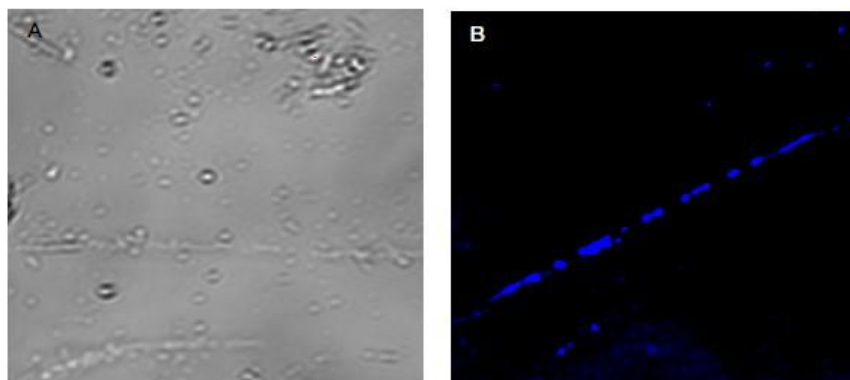
**Figure 5B & 5C** (B) Schematic of protein binding causing CNT attenuation. (C) Diagram of implanted glucose-monitor capillary (Barone *et al.*, 2005).

Alternatively, attempts are also being made to combine the properties of CNT and gold for use in *in vitro* diagnostics (Lint *et al.*, 2009). Gold nanoparticles have biocompatible properties, so they may provide a suitable environment for the exceptional electronic properties of nanotubes. Electrochemical immunosensors for determination of tumor markers are being developed which incorporate gold nanoparticle and CNT as the biosensing platforms. The Au-CNT matrix is used as immobilization substrate for specific marker proteins. Similar to the functionalizing CNT or gold nanocages, immobilization of proteins, antibodies or antigens involves their binding to specific functionalized sites on the surface of the gold nanocages or CNT. This is akin to the binding of glucose to an analyte derivative in the blood sugar biosensor. Immobilization does not require direct binding to the inorganic substrate, rather immobilized molecules are bound to the functionalized groups of the inorganic substrate. For gold-CNT composites, the nano-matrix also contains chitosan (a biopolymer) with reactive amino and hydroxyl and functional groups (Lint *et al.*, 2009).

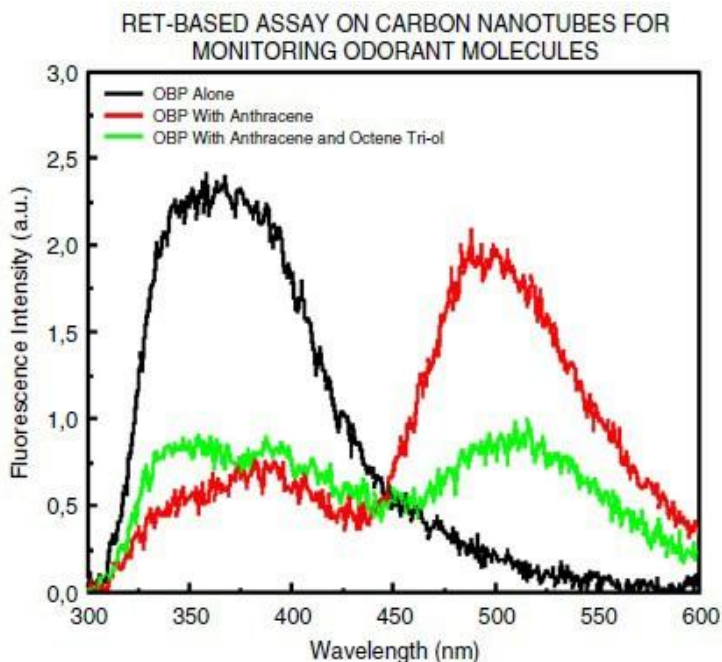
Ultimately, the technology for combining organic and inorganic materials to integrate nano-properties is not restrained to medical applications. Another type of biosensor is playing a major role in the defense and security community. Just as the functionalization of CNT allows for rapid detection of compounds within the body, these nanohybrids can also be used for chemical detection in the environment. Nano-biosensors can be used as odor detectors in varied applications. Odorant-binding proteins, OBP, are extra cellular proteins that play a role in odor detection by carrying, deceiving or selecting odorant molecules (Ramoni *et al.*, 2008). Like the polyaniline polymer wrapped around CNT, the odorant proteins can be immobilized onto CNT. OBP can be attached to CNT by simple incubation of the CNT-OBP at room

temperature for 10 minutes in aqueous buffers (phosphate buffer). The attachment of these proteins to CNT produce sensors which detect the functional groups that will bind to the OBP receptors. The molecule 1-aminoanthracene, AMA, binds into receptor sites on OBP. AMA is found in compounds commonly utilized in the preparation of explosives or produced by explosions (Ramoni *et al.*, 2008). Sensing AMA and similar compounds is of necessity to national security interests, and the functionalized surfaces of CNT can easily detect and they respond through fluorescence attenuation.

Nanotubes functionalized with OBP have specific emission spectra that will change when AMA is attached. Figure 6A shows the optical microscope images of CNT-OBP alone. When AMA is adsorbed on the surface, Figure 6B shows a blue emission. In the presence of AMA, the CNT-OBP hybrids become completely blue in color. Figure 7 shows the emission spectra of CNT-OBP alone (black line), upon addition of AMA (red line) and upon addition of an excess the natural ligand of OBP (green line). The natural ligand of OBP is the baseline response, but when the ligand is removed for replaced, dramatic changes in the fluorescence spectra occur (Ramoni *et al.*, 2008).



**Fig. 6.** Optical microscope images of CNT-OBP without (A) and with (B) AMA immobilization (Ramoni *et al.*, 2008).



**Figure 7.** Fluorescence intensity vs. wavelength spectra for CNT-OBP with different ligands showing fluorescence shift with addition of AMA (Ramoni *et al.*, 2008).

By just adjusting the surface chemistry of CNT, as shown in Figure 7, the optical properties change dramatically. The spectacular change in properties due to adsorbates is the essential key for exploiting the potential of nanohybrids. The properties of nanomaterials, specifically optical properties, are unattainable in conventional biomaterials; therefore, the combination of nanostructures and organic compounds can produce exceptional tools. As illustrated, nanohybrids can be used in an array of biosensor applications. Nanohybrids offer a way to interact with and control complex biological systems by using organic building blocks in conjunction with man-made nanomaterials.

## 2. CONCLUSION

In this reviewing article we described that organic-inorganic hybrid materials are not only a creative alternative to designing new materials and compounds for academic research, but their improved or unusual characteristics allow the development of innovative industrial applications. Today, most of the hybrid materials that have already entered the market are synthesized and processed using conventional soft chemistry based circuits

developed in the 1980s. As far as the future is concerned, there is no doubt that these new generations of hybrid materials, born from very fruitful activities in this field of research, will open up a land of promising applications in many fields: optics, Electronics, ionics, mechanics, energy, environment, biology, medicine for example as membranes and separation devices, functional smart coatings, fuel and solar cells, catalysts, sensors, etc.

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## POSSIBLE UTILIZATION OF ZEOLITES FROM MUNELLA REGION (ALBANIA) FOR BIOSENSORS CREATION

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### ABSTRACT

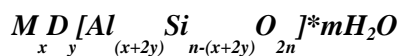
The naturally occurring zeolites are an important group of minerals for industrial activities and other purposes. Zeolites are very stable solids that resist several kinds of environmental conditions that challenge many other materials. They are famous for their variety of applications in different fields due to their special characteristics such as cation exchange and adsorptive capacity. As nanomaterial, they are widely used in energy, environmental, medicine, material science, agricultural, biosensors, catalysis etc. Thus, they have a powerful impact in quality of life and are important for a sustainable economic development. The results of this paper on the mineralogical and chemical composition of Munella zeolites (Northern Albania) and their use for the improvement of poor sandy soils come from the PhD Thesis of Beqiraj (2005). The achievements of the experiment of their use in agriculture were further advanced in the project "Use of zeolites from Munella for the improvement of sandy soils properties", supported by UPT.

**Keywords:** Zeolite, Munella region, biosensors

### I- GENERAL INFORMATIONS ON ZEOLITES

Zeolites are hydrated aluminosilicate minerals made from interlinked tetrahedrons of alumina ( $\text{AlO}_4$ ) and silica ( $\text{SiO}_4$ ). In simpler words, they're solids with a relatively open, three-dimensional crystal structure composed of aluminum, oxygen, and silicon, with alkali or alkaline-Earth metals (such as sodium, potassium, and magnesium) plus water molecules trapped in the gaps between them. Zeolites form many different crystalline structures, which have large open pores (sometimes referred to as cavities in a very regular arrangement and roughly the same size as small molecules (Fig.1).

The general formula of a natural zeolite is (Gottardi & Galli, 1985), (IMA, 1998):



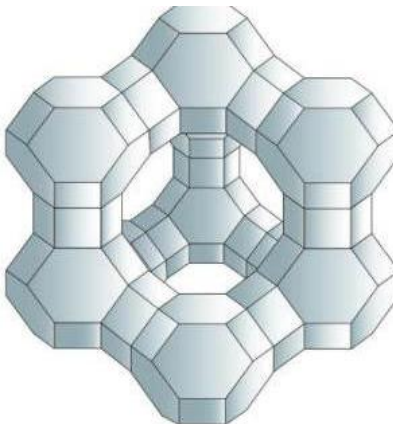
where:

M: Na, K or other monovalent cations

D: Mg, Ca, Sr, Ba or other divalent cations

Zeolites are classified based on different criterions [3]:

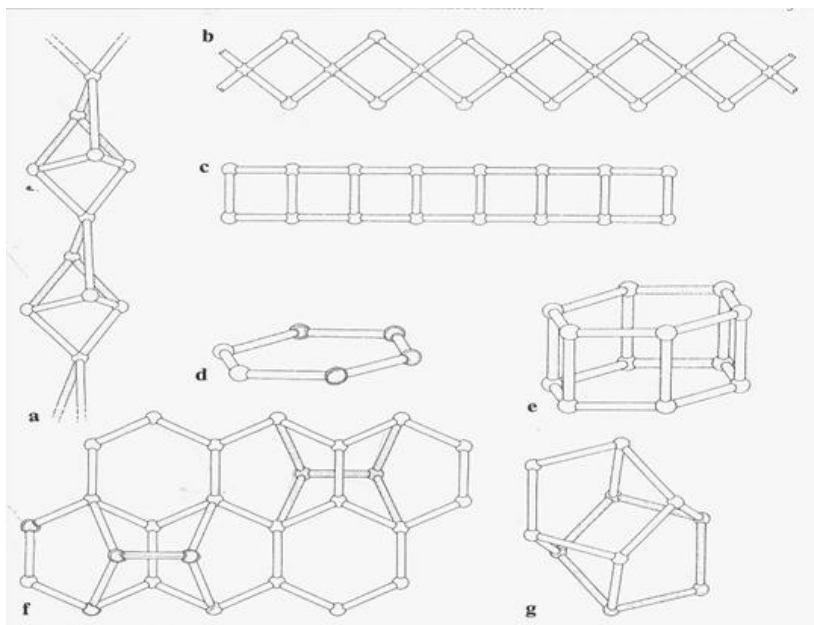
- 1- Structural peculiarities with common SBU of the zeolite framework.
- 2- Order/Disorder distribution of Si, Al ( $R=Si/Si+Al+Fe$ ).
- 3- Type of cation sites in zeolite framework.
- 4- Type of channels and cavities



**Fig.1-** Zeolite structure (Meier W.M., 1968)

The structural units, finite or infinite, may be used to assemble the frameworks of zeolites (Gottardi & Galli, 1985), (Meier W.M., 1968)(Fig.2)





**Fig.2-** a) The structure of fibrous zeolites; b) The singly connected 4-ring chain; c) The doubly connected 4-ring chain; d), e) The 6-ring, single and double; f) The hexagonal sheet with handles; g) The 4-4-1 heulandite unit.(Gottardi & Galli, 1985).

## I.1- Properties and application of Zeolites

The sizes and shapes of the pores are responsible for many of the important applications of zeolites in which they can act as catalysts, molecular sieves and ion-exchange materials – i.e. it is the holes in the structures that lead to their interesting properties!

### **Zeolites in catalysis**

A zeolite can act as a catalyst for a chemical reaction i.e. it can speed up the rate of the reaction without being chemically changed at the end of the reaction. The chemical reaction takes place at catalytic sites within the pore system of the solid. The shape and size of the pore system therefore determines which reactions can take place in a particular zeolite.

### **Zeolites as molecular sieves**

Because dehydrated zeolites have very open porous structures, they have large internal surface areas and are capable of adsorbing large amounts of substances. This property, together with their ability to adsorb molecules of

certain sizes or shape whilst excluding others (shape selectivity again) has led them to be used as ‘molecular sieves’.

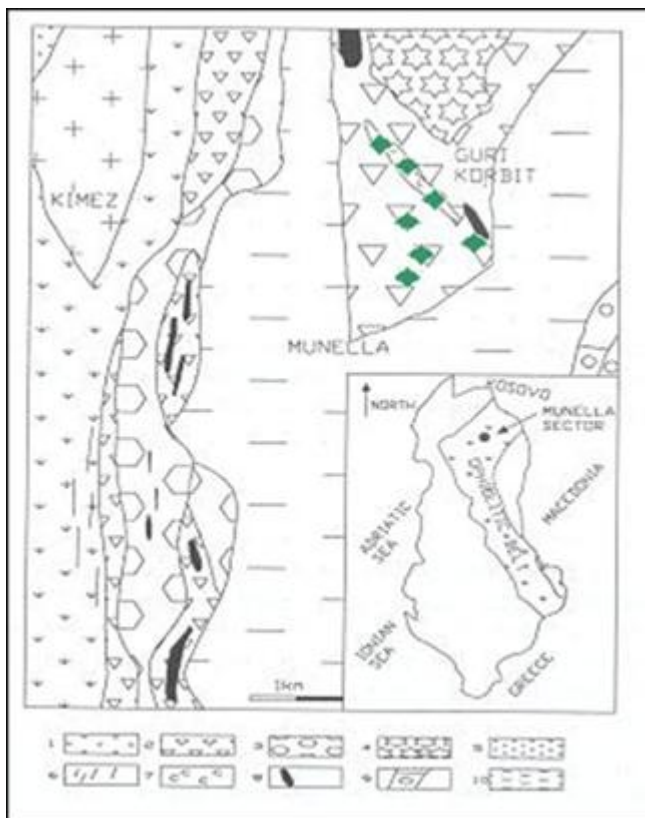
### **Zeolites as ion-exchange materials**

The alkali-metal cations in zeolites are only loosely held in the pores and they can often readily be exchanged for other types of metal when placed in aqueous solution. This was mentioned above for the cleaning up of radioactive waste. A more widespread use is in water softeners.

## **II- NATURAL ZEOLITES FROM MUNELLA REGION, ALBANIA**

The ophiolitic formations of Munella, which belong to eastern (SSZ) – type ophiolites, are mostly composed of volcanic rocks and subordinate plutonic ones. The plutonic rocks that outcrop in the north-western part of region consist of quartz diorites and plagiogranites. The volcanic sequence consists of pillowed to massive flows ranging in composition from basalt and basaltic andesite in the lower sections to andesite, dacite, and rhyolite-dacite in the upper part (Shallo M., 1991),(Beccaluva L. et al., 1994).

Zeolitic mineralization occurs as separated 2-3 to 20-30m thick layers that are concordant with rhyolite-dacite hosting rocks and has sharp contacts with them. They are green to brown coloured, massive rocks, without any obvious stratification and very homogenous. The interlayering of zeolitic mineralization with rhyolite-dacite rocks and especially the presence of quartz and albite phenocrysts and albite-quartz spherulites similar with those observed in the microcrystalline mass of rhyolite-dacitic rocks, favour the opinion that the zeolite mineralization derived by an intensive metasomatic replacement of the microcrystalline mass of rhyolite-dacite rocks (Beqiraj Goga), E., 2005) (Fig.3).



**Fig. 3-** Geological map of Munella region (1:50000). 1-Basalt; 2- Andesite; 3- Dacite; 4- Rhyolite; 5- Plagiogranite; 6- Sheeted Dyke; 7- Zeolitic rocks; 8- Sulphide Sulphide mineralization; 9-Limestones (Cr1); 10-Melange (J3t – Cr1); rhomb – sample sites (Beqiraj Goga, 2005).

## II.1- Methods of study

Natural zeolites from Munella area (Central Mirdita, Albania) are used in this study. Samples were taken from outcrops, drilling cores and galleries (Fig.3).

After processing of zeolitic materials bulk and separated samples were examined by XRD, polarized OM, SEM, EMPA, TG/DTA and IR. Analyses were performed at Orleans University (France) and University of Rome “La Sapienza” (Italy)

The experiment of their use for the improvement of poor sandy soil properties was accomplished in green house of the Agricultural University of Tirana (Beqiraj Goga et. al., 2006).

### III- CHARACTERISTICS OF ZEOLITES FROM MUNELLA, ALBANIA.

The microscopic study in combination with microprobe analyses and XR diffraction, revealed the following mineralogical composition of zeolitic rocks: zeolites (75-80%), quartz, chlorite, carbonate, euhedral plagioclase (albite) and pyrite phenocrysts (Fig.4,5a,b).

The XRD data and microprobe analyses indicate that the zeolite minerals can be classified to the “Heulandite” group, of “stilbite” family and are present as a Stilbite-Stellerite solid- solution (SS), showing a Ca-rich trend. SS probably coexists with heulandite. These interpretations are compatible with TG/DTG and DTA results. Scarce laumontite and mordenite are present as vein and vesicle fillings (Beqiraj (Goga), E., 2005).

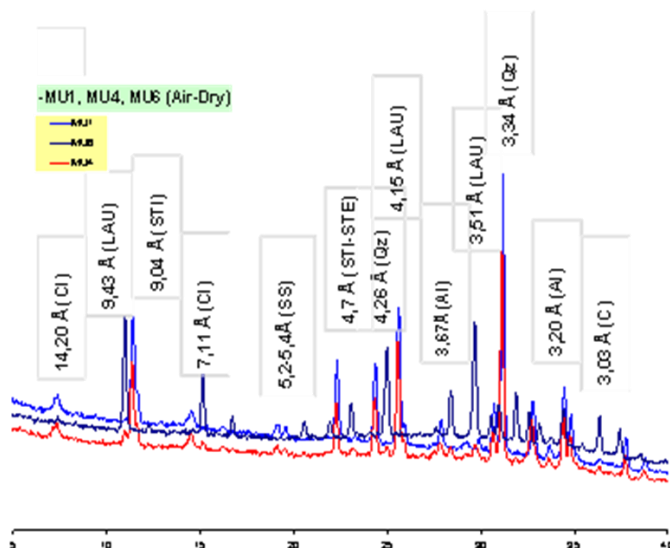
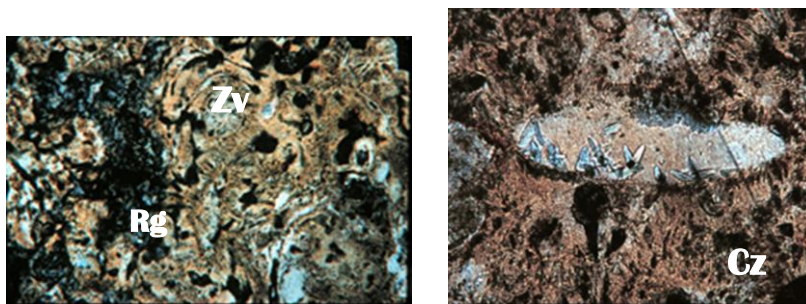
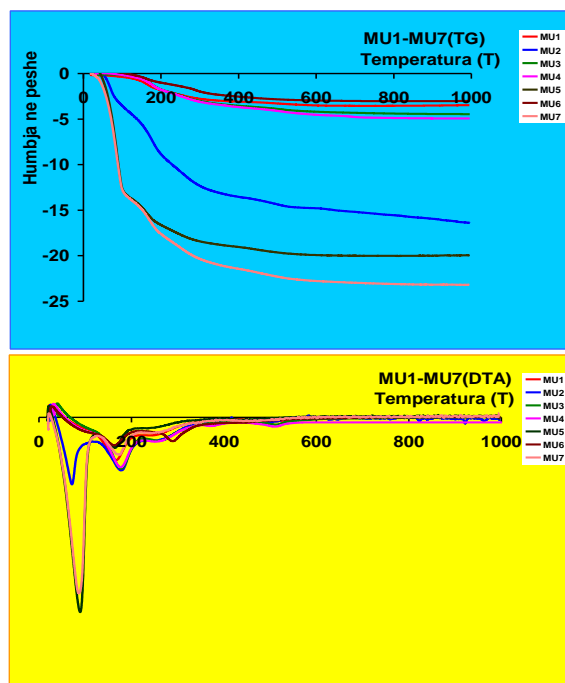


Fig. 4-X-ray diffraction pattern of MU1, MU4 and MU6.



**Figure 5.** Microphotographs of zeolite-bearing rocks. (Rg=residual glass; Cz=zeolitic cement; Zv=vesicles with zeolites; magnification: x 48).

Zeolitic material show the reversible dehydration during three steps (Fig. 6), respectively at 75-80°C, 170-175°C and  $\approx 250^\circ\text{C}$ . Removal of zeolitic water is associated by an endothermic reactions. Other peaks at  $\approx 500^\circ\text{C}$  and  $\approx 600^\circ\text{C}$  belongs to removal of hydroxyl groups. Immediately after hydroxyl groups have been removed, the dehydroxylated process is accompanied by structural changes.



**Fig. 6-** Thermal curves of Munella zeolitic material

Ion-exchange behaviour of zeolitic material, based on methods by Collela et al. (1982), Pansini (1996), Capelleti et al. (1999), consists in: a cation exchange capacity  $CEC = 1.9 - 2.42 \text{ meq/g}$  showing the following selectivity for cations:  $Zn^{2+} < Cu^{2+} < Pb^{2+}$  (Fig. 13 ) (Beqiraj Goga, E., 2005).

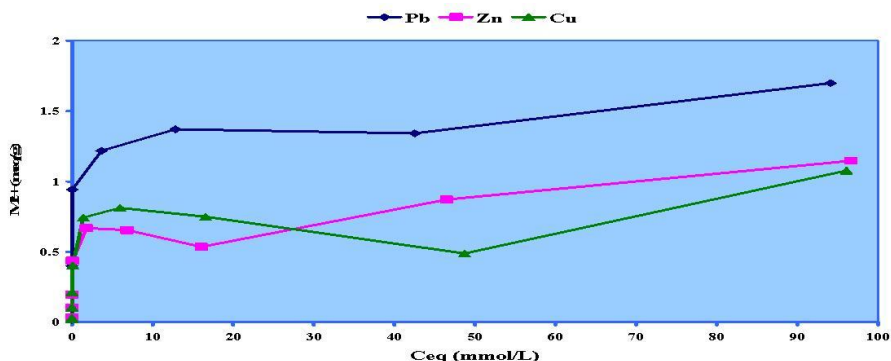
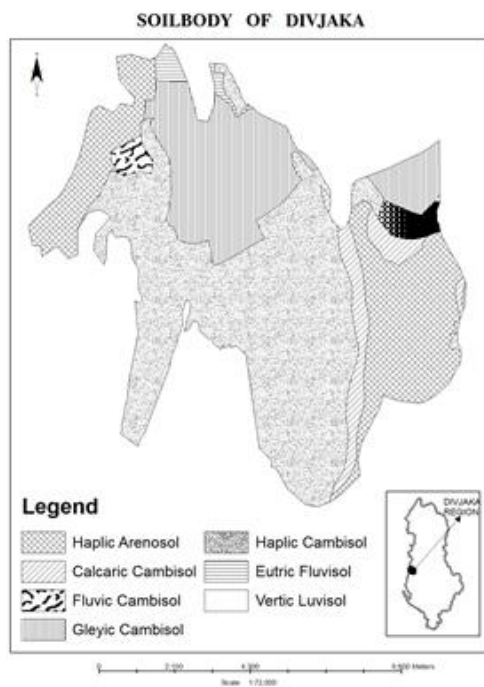


Fig. 7- Selectivity isotherms for Pb, Zn, Cu of Munella zeolitic material.

#### IV- APPLICATION OF ZEOLITES FROM MUNELLA REGION IN AGRICULTURE

The zeolitic material used in the experiment has been sampled from the Munella region (Beqiraj Goga E. et al., 2008). Zeolitic material was ground to 100 mesh. This material was analysed for the chemical characteristics by the X-/fluorescence, in Activation Laboratories LTD, Canada.

The application of zeolites was made on a sandy soil (Haplic Arenosol, (Gjoka, F & Cara K. 2003) coming from Lushnja District (Fig.8). This soil type (known as arenosoil) is considered as a problematic one because of its unfavourable properties for plant growth. Total surface of arenosoils in Albania is estimated at about 10. 000ha. this surface is under agricultural production.



**Fig.8-**Pedological map of Divjaka region (Albania) (Beqiraj Goga, 2005)

The experiment was conducted in a randomized block with four repetitions. The experiment scheme was performed in seven pots, where different combinations of NPK-natural zeolite were tested (Fig.9).



**Fig. 9-** Photo of the experiment.

Where:

POT -1 Control

POT -2 NPK (148:75:60) (kg/ha a.s. N, P and K)

POT -3 (Z - 600 kg/ha)

POT -4 (Z - 1200 kg/ha)

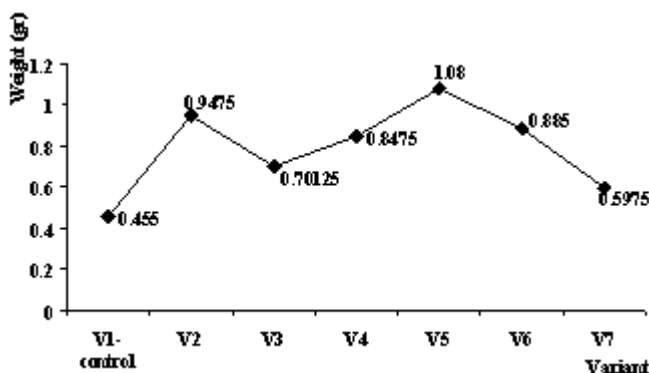
POT -5 (Z - 600 kg/ha + NPK - 148:75:60)

POT -6 (Z - 1200 kg/ha + NPK - 148:75:60)

POT -7 (Z - 5 % of soil weight)

***Effect of zeolitic material in indexes of dry plant matter (Relative Agronomic Efficiency).***

**Relative Agronomic Efficiency (RAE)** is referred to quantity in weight of dry plant material. As it can be seen in the fig.10, Relative Agronomic Efficiency (RAE) of zeolitic material, used as pure zeolite or in combination with chemical fertilizer, considering the productivity of dry matter in pot V2 (NPK) as 100%, was ranged as follows: V7 (67.51%) < V6 (81.94%) < V3 (88.13%) < **V2- NPK (100%)** < V4 (101.50 %) < V5 (127.43%).

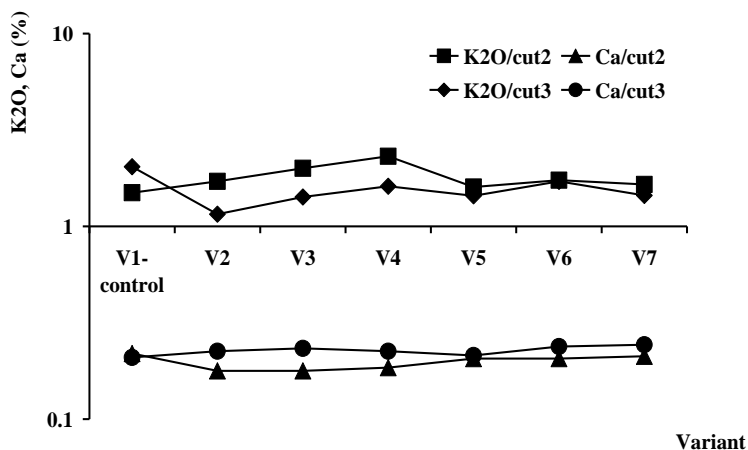


**Fig.10-** Variation of weight of dry plant matter by variant

***Effects of zeolitic material in indexes of essential nutrients in plant (efficiency of essential nutrients)***

As shown in figure 11, the increase of Ca content in plant was accompanied by the corresponding decrease in K content, probably due to the cation exchange reactions in the zeolite. The highest content of Mn and Cu, determined in V1- control variant, may be dedicated to the rate of selectivity for cations  $Zn^{2+} < Cu^{2+} < Pb^{2+}$  of Munella zeolite. So, Zn element can be up taken by plant more easily than Cu (fig. 7) which is probably fixed in porous structure of zeolite. Various cations interact differently with various zeolite framework and their associated electrical fields.





**Fig. 11-** Variation of K<sub>2</sub>O, CaO (%), second and third harvest, in dry plant matter.

The highest content of Mn in the variant V1 may be due to high binding capacity of Fe - Mn oxides and aluminosilicates for metals. Thus, the immobilizing capacity of MnO<sub>2</sub> was higher than that of other metals. These effects were assumed to be related to immobilization of metals due to formation of insoluble metal-organic complexes and increased cation exchange capacity (CEC) (Singh B. R. & Oste L., 2001).

The presence of zeolites ameliorates the physical and chemical quality of soil and maintains the infiltration rates of soil and soil moisture. By way of its properties, zeolitic material can reduce nutrient loss due to leaching by increasing the retention of nutrients and slowly releasing them as needed by soil and plants.

The best performance of zeolite application was the treatment with 600 kg/ha zeolite (101.50%) and treatment 600 kg/ha zeolite + NPK (127.43%).

The use of zeolite combined with fertilizer ensures equilibrium conditions for main indexes of soil fertility (humus, N, P, Cu, Mn and Fe), at the end of the experiment.

The report zeolite/fertilizer, in the combined treatments, must be carefully considered according to the case.

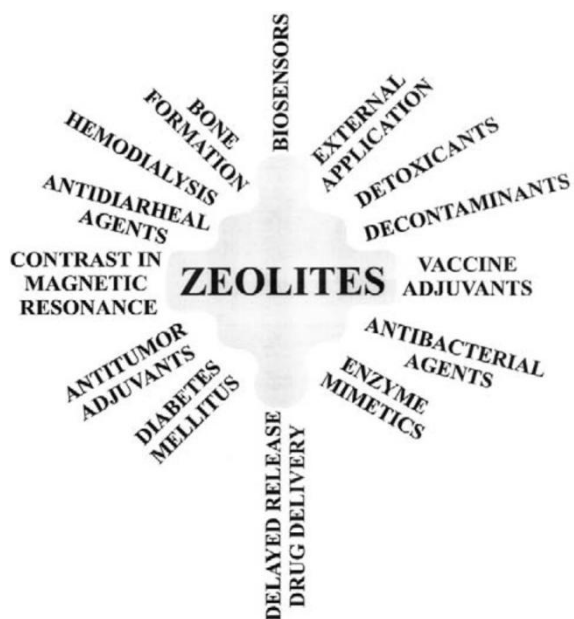
## **V- POSSIBLE APPLICATION OF MUNELLA ZEOLITES FOR BIOSENSORS CREATION**

Last years, isexperimented possible utilization of natural zeolites for different biosensors creation.

Different modifications of the zeolites Na<sup>+</sup>-Beta and LTA were applied for improving the working characteristics of a urea biosensor. The bio selective

membrane of the biosensor was based on urease and different zeolites co-immobilized with bovine serum albumin on the surface of a pH-FET(O. O. Soldatkin et al., 2013).

According to Kresimir Pavelic' & Mirko Hadzija, 2003, zeolites are widely used in medicine (Fig. 12), such as for controlled release of agents against microbial pollution; prevent and eliminate organopoinsoning; heavy metals released in wastewater are among the most worrisome pollution problems due to their cumulative effects along the food chain.



**Fig. 12-** Different uses of zeolite in medicine  
[Kresimir Pavelic' and Mirko Hadzija, 2003]

Based on chemical - physical properties of zeolitic material from Munella region and on experimental data for agricultural uses, we can say that this material could be experimented for biosensors creation, as well.

## CONCLUSIONS

The following conclusions could be drawn: i) mineralogical composition of zeolitic rocks consists of zeolites (75-80%), quartz, chlorite, carbonate, euhedral plagioclase (albite) and pyrite phenocrysts, ii) zeolite minerals can be classified to the "Heulandite" group, of "stilbite" family and are present as

a Stilbite-Stellerite solid- solution (SS), showing a Ca-rich trend. SS probably coexists with heulandites, iii) the presence of zeolites ameliorates the physical and chemical quality of soil and maintains the infiltration rates of soil and soil moisture, iv) the use of zeolite combined with fertilizer ensures equilibrium, v) conditions for main indexes of soil fertility (humus, N, P, Cu, Mn and Fe), at the end of the experiment and, vi) based on chemical and physical properties of zeolitic material from Munella region and on experimental data for their agricultural uses, we may assume that this material could be also experimented for possible biosensors creation.

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## BULK NANOSTRUCTURED MATERIALS PRODUCED BY SEVERE PLASTIC DEFORMATION PROCESSING

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### ABSTRACT

During the past decade, fabrication of bulk nanostructured metals and alloys using severe plastic deformation (SPD) processing, has been evolving as a rapidly advancing direction of nanomaterials science, aimed at developing materials with new mechanical and functional properties for advanced applications. Several different SPD processes are now available, but the most versatile and the most appropriate for use in industrial applications, appears to be Equal-Channel Angular Pressing (ECAP), Accumulative Roll-Bonding (ARB) and High-Pressure Torsion (HPT). These SPD methods lead to very significant grain refinement to the submicrometer or even the nanometer level and have been widely used for a large range of metals and alloys. In the present work, we present an overview of the most used methods of severe plastic deformation for grain refinement and the production of bulk nanomaterials with enhancement in their mechanical and functional properties. In order to examine the potential for using ECAP to refine the grain size and improve the mechanical properties, the commercial 5754 Al and 3004 Al alloys, were selected for study. Processing by ECAP gives a reduction in the grain size and an increase in the microhardness of the alloys, and in the 0.2% proof stress, by a factor of three times.

**Keywords:** nanostructured materials; severe plastic deformation; equal channel angular pressing (ECAP); mechanical properties.

### 1. INTRODUCTION

Grain size reduction is one of the most attractive ways for improvement of mechanical properties of metallic materials. The properties of a metal are strongly influenced by the grain size and the well-known Hall-Petch equation which predicts an increase in yield stress ( $\sigma_y$ ) with a decrease in grain size ( $d$ ) has been shown to be applicable to a wide variety of metals.

$$\sigma_y = \sigma_0 + kd^{1/2} \quad (1)$$

It follows from Eq. (1) that the strength increases with a reduction in the grain size and this has led to an ever increasing interest in fabricating

materials with extremely small grain sizes (Segal, 1999; Valiev and Langton, 2006; Zhilailiev and Langton 2008; Hohenwater *et al.*, 2009). One of the reasons for the current interest in very fine grained metallic materials is the concept that control of mechanical properties by processing may be more desirable than the practice of controlling properties by alloying. It is necessary to define some basic terms. Using the definitions developed in the literature (Valiev and Langton, 2006; Hohenwater *et al.*, 2009; Ruppert *et al.*, 20015), bulk ultrafine-grained (UFG) materials are defined as bulk materials having homogeneous and reasonably equiaxed microstructures with average grain sizes less than 1  $\mu\text{m}$  and with a majority of grain boundaries having high angles of misorientation. These UFG structures divide into materials having submicrometer grain sizes within the range of 0.1 – 1  $\mu\text{m}$  and true nanocrystalline materials where the grain sizes are < 100 nm. Now is used the more general term “bulk nanostructured materials”. Due to their refined microstructure (small grain size) they provide attractive properties, that cannot be achieved in conventional materials with the same chemical composition (Zhilyaev and Langton, 2008; Hohenwater *et al.*, 2009; Ruppert *et al.*, 20015; Furukawaka *et al.*, 2001; Valiev *et al.*, 2006).

It is now established that bulk nanostructured materials may be fabricated using two approaches, which are termed the “bottom-up” and “top-down” procedures (Segal 1999; Valiev and Langton 2006). In the “bottom-up” procedure, the bulk solids are fabricated through the assembly of individual atoms and examples of this approach include inert gas condensation, electrodeposition and ball milling with subsequent consolidation. Although these approaches are able to produce materials with small grain sizes, they have disadvantages because the sizes of the finished products are always very small, there may be some contamination introduced during processing and there is a low level of residual porosity. These difficulties have prevented these methods for reaching larger practical applications (Valiev and Langton, 2008).

The “top-down” approach avoids the introduction of either contaminants or porosity by taking a bulk solid with a relatively coarse grain size and then processing it to refine the grain size to the submicrometer level. In recent years, as a versatile alternative, a “top-down” method called Severe Plastic Deformation (SPD) has gained importance because of the direct conversion of bulk metals and alloys with conventional grain sizes to nanoscaled materials with outstanding new properties. In order to convert a coarse-grained solid into a material with ultrafine grains, it is necessary both to impose an exceptionally high strain in order to introduce a high density of dislocations and for these dislocations to re-arrange to form an array of grain boundaries. Specifically, SPD processing is defined as any method of metal forming in which a very high strain is imposed on a bulk solid without the introduction of

any significant change in the overall dimensions of the sample and having the ability to produce exceptional grain refinement (Zhilyaev and Langton, 2008; Hohenwater *et al.*, 2009; Ruppert *et al.*, 20015; Vevečka *et al.*, 2001; Furukawaka *et al.*, 2001; Valiev *et al.*, 2006; Horita *et al.*, 2000; Izairi *et al.*, 2013; Prell *et al.*, 2008).

The aim of this work is to present an overview of the most used methods of severe plastic deformation for production of bulk nanostructured materials with significant enhancement in their mechanical and functional properties. In this work, we report also how inducing SPD by equal channel angular pressing (ECAP) can produce a refinement of the grain size and improve the mechanical properties of two commercial 5754 Al and AA3004 alloys.

### Methods for Nanostructured Materials by SPD

There are three major procedures now being developed in SPD processing. The first, known as High Pressure Torsion (HPT), involves subjecting a sample, in the form of a thin disk, to a high pressure and concurrent torsional straining (Fig.1) (Zhilyaev and Langton 2008; Hohenwater *et al.*, 2009). The second is Accumulated Roll Bonding (ARB), where a sheet is rolled, cut in half, stacked to double the height, rolled again, cut in half again, etc. (Fig.2) (Ruppert *et al.*, 2015). The third procedure known as Equal Channel Angular Pressing (ECAP), which is the most promising SPD technique, involves pressing a bar or rod through a die within a channel bent into an L-shaped configuration (Fig.3). ECAP can subject a metal work-piece to arbitrarily large shear strain under high pressure without changing its dimensions and so the work-piece is repeatedly pressed through the same die. For an ECAP die with an angle  $90^\circ$  (Fig.3), each processing pass introduces a von-Mises strain of 1.1. [6-10]. An important merit of ECAP is its potential to be scaled-up for industrial applications (Horita *et al.*, 2000; Cabibbo, 2013).

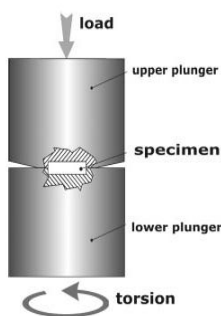


Fig. 1: Schematic

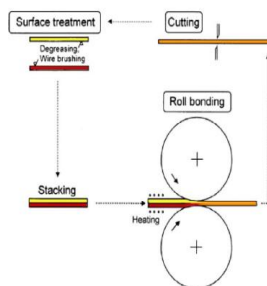


Fig. 2: Sketch of ARB technique

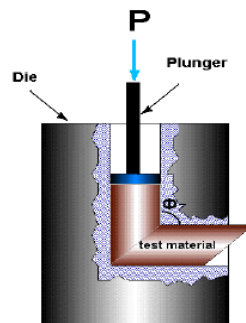


Fig. 3: Schematic view of HPT set-up an ECAP die

## Improvement of mechanical properties of commercial Al alloys processed by ECAP

### *Experiment*

In this investigation, experiments were conducted on two commercial aluminum alloys AA 5754 and AA 3004. AA 5754 has the following composition in wt.%: 2.4-2.6 % Mg, 0.1-0.6 % Mn, 0.4 % Cr, 0.4 % Fe, 0.4 % Si, 0.2 % Zn in the overall Al. The mentioned alloy was used for producing automotive parts. Observations by optical microscopy revealed grain size of about 70  $\mu\text{m}$  in the as received condition.

The other studied alloy was AA3004 whose composition (wt.%) is as follows: 1.09Mn, 1.08Mg, 0.55Fe, 0.20Si, 0.19Cu, 0.01V, 0.01Ti, in the overall Al. The 3004 aluminium alloy is widely used in the container, packaging, and automobile industry, because of its excellent specific strength, corrosion resistance and formability (Vevečka *et al.*, 2013; Izairi *et al.*, 2013; Prell *et al.*, 2008). Observations by optical microscopy revealed grain size of about 50  $\mu\text{m}$  in the as received condition.

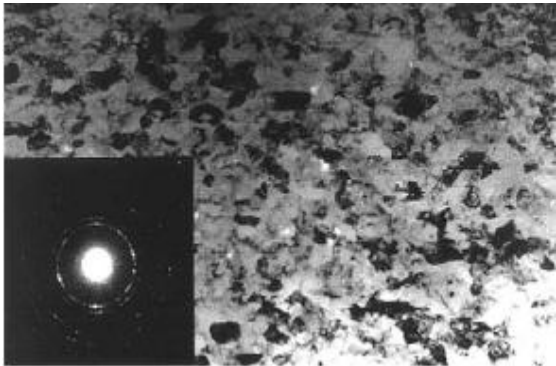
The material was received in the form of cylinders having diameters of 10 mm and with 10 cm in length. The samples were subjected to ECAP, up to a total of 7 passes through the ECAP die for AA 5754, equivalent to an imposed strain of  $\sim 6$ , and up to 6 passes for AA 3004. All processes were performed at room temperature and each billet was pressed using route  $B_C$  where the sample is rotated in the same sense by  $90^\circ$  around the longitudinal axis between each pass (Valiev and Langton 2006; Furukawa *et al.*, 2001). Experiments have shown that route  $B_C$  the optimum procedure for most rapidly attaining an array of equiaxed grains separated by high angle boundaries (Valiev and Langton 2006; Furukawa *et al.*, 2001). Small pieces were cut from the as-pressed cylinders. Each billet was polished to a mirror-like finish and the Vickers microhardness, HV, were taken on the surface of each sample using a HSV- 30 SHIMADZU microhardness tester, using a load of 100 g, applied for 15 s. Strength and ductility were measured by uniaxial tensile tests at room temperature, using a testing machine, with an initial strain rate of  $3 \times 10^{-3} \text{ s}^{-1}$ .

## 2. RESULTS AND DISCUSSIONS

Microstructural examinations by electron microscopy of AA 5754 samples pressed through 2 to 6 passes, performed in a previous work (Vevečka *et al.*, 2001), revealed an array of reasonably equiaxed grains having average sizes of  $< 1 \mu\text{m}$ . An example of the as pressed microstructure is shown in Fig.4, after 4 passes, where measurements indicated average grain sizes of  $\sim 0.3$ - $0.4$

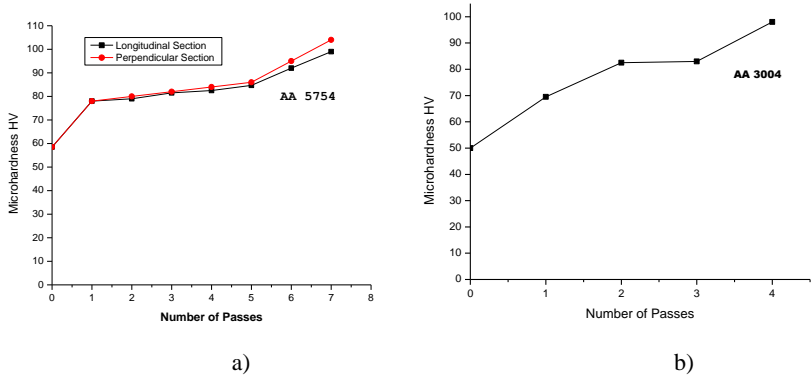


μm, demonstrating that ECAP is an effective procedure for attaining an ultrafine grain size.



**Fig. 4** Microstructure of AA 5754 observed by TEM after ECAP through 4 passes.

The measurements of the microhardness values are plotted in Fig. 5 for AA 5754 where measurements were taken on 2 orthogonal planes and the first point (zero passes) refers to the unpressed alloy. Two conclusions may be reached from this plot. First, the hardness is essentially independent of the plane of sectioning. Second, the value of hardness increases by approximately a factor of two after a single pass and thereafter increases slowly with additional passes. Similar results are attained also for the AA 3004 (Fig. 5 b).



**Fig. 5:** Microhardness Hv versus number of passes in ECAP

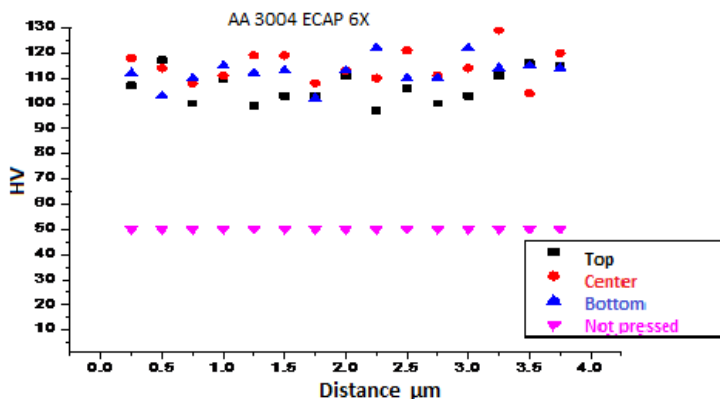


Fig. 6: Micro-hardness plots after 6 passes on the cross-sectional plane for the top, central and bottom positions.

On the cross-sectional plane of billet of AA 3004 after 6 passes, three traverses were made at distances of 2.0 mm from the central line and for each separate traverse, the individual measurements were recorded in incremental steps of 250  $\mu\text{m}$ . The individual microhardness measurements were plotted against the position and the microhardness of the material in the unpressed condition was also measured to provide a comparison with the as-pressed billet (Fig. 6). The results show that the microhardness evolve in a consistent manner; there is an essentially homogeneous structure throughout the billet.

Strength and ductility were measured by uniaxial tensile tests and the resulting engineering stress-strain curves are shown in Fig. 7. The results show that the elongations to failure are very much reduced after ECAP in comparison with the sample in the as received condition. Thus, the unpressed alloy pulls out to an elongation  $\sim 35\%$ , but after pressing from 1-6 passes, the elongations are reduced to  $\sim 10\%$ . It is apparent from Fig. 7 that the value of 0.2% proof stress increases by a factor  $\sim 3$  times from  $\sim 80$  MPa in the as-received alloy to  $\sim 240$  MPa after a single pass in ECAP and thereafter for additional passes the increase is relatively minor.

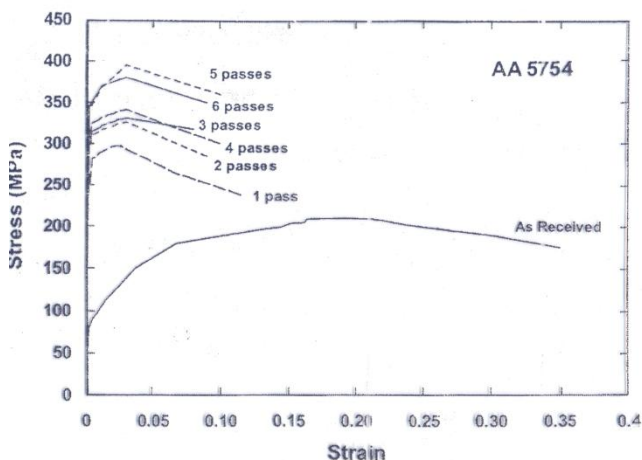


Fig. 7 Stress versus strain at room temperature for AA5754.

### 3. CONCLUSIONS

Samples of commercial AA 5754 and AA3004 alloys were processed by ECAP at room temperature through 1-7 passes through the ECAP die. It was demonstrated that the reduction in the grain size leads to a significant improvement in the strength of the studied alloys at ambient temperatures. The average microhardness values increase by approximately a factor of two, for both alloys. The values of HV were recorded on cross-sectional plane along three traverses after six passes for AA 3004 and the hardness values are almost homogeneously distributed through the cross-sectional plane. For AA 5754 stress-strain curves show an increase in the 0.2% proof stress by a factor of at least three times. In addition, the measured elongations to failure are reduced from ~ 35% at room temperature in the unpressed alloy to ~ 10% after ECAP.

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